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Biology and Control of Currant Lettuce Aphid *Nasonovia ribisnigri*

Gemma Louise Hough

A thesis submitted in partial fulfilment of the requirements for the degree of:

Doctor of Philosophy in Plant and Environmental Sciences

School of Life Sciences, The University of Warwick

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A key to the abbreviations used in this study can be found in the Appendix (Table A1).

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Acknowledgments

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I would like to thank the Horticultural Development Company for funding this research and the opportunities they gave me to gain experience of the horticultural industry which has helped me in beginning my career.

Declaration

This thesis contains my own work which has not been submitted for a degree at another university

Some data gathered for this thesis were presented in the following paper:

Hough, G., & Collier, R.H. (2013) The biology and control of the currant lettuce aphid *Nasonovia ribisnigri. Integrated Protection of Field Vegetables IOBC/WPRS Bulletin.* In Press

Summary

There is a consensus that the development of successful Integrated Pest Management strategies requires a detailed understanding of pest biology. In the case of the currant lettuce aphid (*Nasonovia ribisnigri*), an economically important pest aphid of lettuce, sources of such information are limited. This study considers key aspects of *N. ribisnigri* biology which influence its control. In particular, it makes comparisons between biotypes which succumb to (wild-type) or overcome (resistance-breaking), the host plant resistance (Nr-gene) in commercial lettuce cultivars.

Experiments on the effects of temperature and photoperiod on the development of *N. ribisnigri* showed no differences between wild-type and resistance-breaking biotypes. At low temperatures (5, 10 and 15ºC), wild-type biotypes developed to adulthood on resistant cultivars, indicating that the Nr-gene is temperature sensitive. A linear regression between development rate and temperature estimated a lower developmental threshold of around 4.7ºC.

Nasonovia ribisnigri usually overwinters as a diapausing egg but overwintering nymphs/adults have been observed. In the laboratory eggs were obtained at 12ºC 13L:11D. Sequential sampling of eggs from the field suggested that diapause ended between late January and early February. Post-diapause development was estimated to take <50 day-degrees using a LDT of 4.7°C.

Nasonovia ribisnigri survived the winter as nymphs/adults on *Veronica arvensis* in the Midlands. Other weed species were suitable hosts in the laboratory: *Chichorium intybus, Crepis capillaris, Lapsana communis, Hieracium aurantiacum, Hieracium pilosella, Veronica spicata* and *Veronica officinalis*.

Field trials, using sequentially planted plots of lettuce, and applying ‘exclusion’ and pesticidal treatments indicated that natural enemies and emigration regulate aphid populations in the summer and contribute to the mid-summer crash.

A large-scale screen of 96 cultivars and wild relatives of lettuce identified new sources of resistance against wild-type and resistance-breaking biotypes.

Results from this study can be used to inform further development of an Integrated Pest Management strategy for this pest.

Chapter 1: Introduction

Aphids as pests

Aphids are small phytophagous phloem-feeding insects which collectively infest a wide range of plant species including over 100 economically important types of crop. While the implications of aphid infestations are not always significant, on certain crops they can considerably reduce crop yield and quality, as a result of direct feeding and indirect damage through vectoring plant pathogens (Blackman and Eastop, 1984).

This research focuses on the currant lettuce aphid, *Nasonovia ribisnigri* (Hemiptera, Aphididae) which is an important arthropod pest of lettuce crops. *Nasonovia ribisnigri* is one of four significant species of pest aphid infesting lettuce, and is the most important due to its preference to feed in the centre of lettuce heads where the infestation is often difficult to control with foliar insecticides, resulting in unmarketable produce and therefore financial losses for growers (Parker, *et al.*, 2002). Rapid population development of *N. ribisnigri* can also lead to stunted plant growth and affect the palatability of harvested lettuce (RIS, No date-a).

Nasonovia ribisnigri is known to transmit gooseberry vein-banding virus on its winter host *Ribes* species and the mosaic diseases of cauliflower and cucumber (Blackman and Eastop, 1984). However, *Nasonovia ribisnigri* appears to be unable to transmit lettuce mosaic virus (Keep and Briggs, 1971; Nebreda, *et al.*, 2004; Moreno, *et al.*, 2007) although one report from the USA contradicts this assumption (Davis, 1997).

Like all aphids, *N. ribisnigri* feed using their stylets which penetrate the plant tissue through an intercellular pathway to allow direct ingestion of phloem sap from the sieve elements (Tjallingii and Esch, 1993). As a result of this removal of cell contents, plants display a range of species-specific symptoms affecting yield and quality (Pettersson, *et al.*, 2007). Aphids also produce honey dew which remains on the leaf surface and can promote the development of unsightly black moulds (Lamb, 1959; Cox, 2004).

Historically, aphids have been controlled by farmers and growers through the application of pesticides. Due however, to recent concerns about potential chemical

residues and the imposition of high selective pressures for insecticide resistance, there have been increased demands for farmers and growers to adopt Integrated Pest Management (IPM) practices (Cuthbertson, *et al.*, 2007). For *N. ribisnigri,* resistant lettuce cultivars are also available but now that these are grown widely, the increased selection pressure appears to have resulted in a new resistance-breaking biotype of *N. ribisnigri* which has overcome the resistance provided by these cultivars (van der Arend, 2003; Smilde, *et al.*, 2009).

Recent research on *N. ribisnigri* has focused on its development, insecticide resistance and its response to resistant cultivars. Therefore, there is little information available on its basic biology which is vital for creating new and informed control strategies.

Biology and behaviour

Classification and identification

Nasonovia ribisnigri (Mosley) is a small soft bodied insect belonging to the family Aphididae which is one of the three families of the super-family Aphidoidea (Table 1.1). The Aphididae family contains approximately 4700 species which utilise a wide range of host plants and display a range of specialised life-cycles (Blackman and Eastop, 2007). Economically important aphid species such as *N. ribisnigri,* are usually found in the sub-family Aphidinae, where in temperate climatic zones it is estimated that one plant species in four is infested by members of this sub-family (Dixon, 1998).

Table 1.1 The classification of *Nasonovia ribisnigri.*

Order	**Sub-order**	**Super-Family**	**Family**	**Sub-family**
Hemiptera	Sternorrhyncha	Aphidoidea	Aphididae	Aphidinae

Aphids possess various features which allow identification to species, of which the main ones include the cauda, siphunculi, head and specific abdominal markings.

Various keys are available to help with the identification of aphids and two were used throughout this research (Tatchell, 2004; Blackman, 2010).

Adults of *N. ribisnigri* can be alate or apterous. Usually, their body colour ranges from several shades of green to yellow and red. Adults also possess conspicuous black dorsal markings which aid their identification. Alate and apterous adults range from 1.5-2.5 mm and 1.3-2.7 mm in body length respectively (Blackman and Eastop, 1984). When looking at the siphunculi, apterae possesses dark tips whilst the siphunculi of alates are all darker (RIS, No date-a). Adult *N. ribisnigri* also possess a prominent central tubercle on their head, between the antennal tubercles (Tatchell, 2004). Figure 1.2 illustrates the key points described for identifying apterous and alate *N. ribisnigri*.

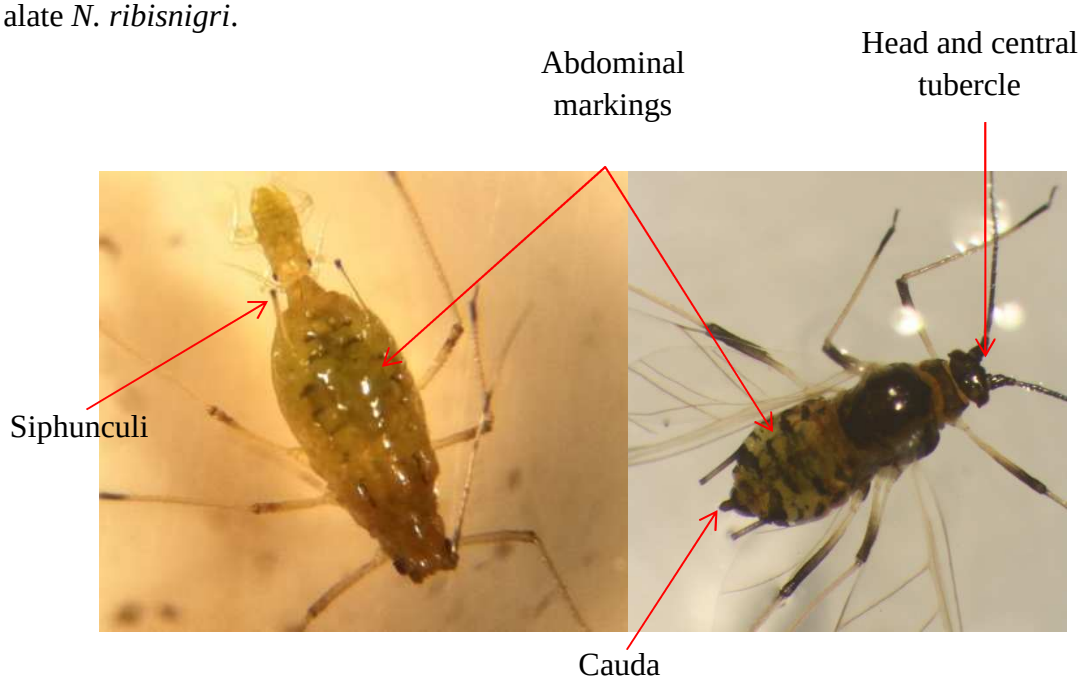


Figure 1.2 *Nasonovia ribisnigri* aptera (left) and an alate parthenogenetic female (right) from the secondary host.

Life-cycle

Aphids display various different types of life-cycles and these are usually adapted specifically to the host plants they infest, ensuring that they are always in synchrony with them and can utilise their resources effectively. *Nasonovia ribisnigri* is a heteroecious holocyclic aphid, meaning it alternates between two host plants during the year and undergoes both asexual and sexual reproduction.

Nasonovia ribisnigri colonises lettuce crops and other species of Asteraceae during the summer, where it reproduces parthenogenetically to produce viviparous offspring (which can develop into apterous or alate adults) to continue exploiting nearby host plants or to found new colonies.

During autumn, in response to decreasing temperatures and shortening photoperiods, the production of alate males and alate gynoparae is stimulated. These migrate to the primary host, *Ribes* species, in particularly blackcurrant (*Ribes nigrum*) and gooseberry (*Ribes grossularia*).

On returning to their primary host, the gynoparae produce egg laying females known as oviparae, with which the males mate. Fertilised eggs are then laid around the bud apex and these spend the winter in diapause, hatching in the spring to produce a fundatrix. The fundatrix is a highly fecund morph which reproduces parthenogenetically and colonises the primary host before producing spring migrants, which move back to the secondary host to begin the summer phase of the life-cycle.

Some species of aphid however, such as *Myzus persicae* and *Rhopalosiphum padi*, possess clones which fail to produce sexual forms and remain as parthenogenetic aphids (anholocyclic clones) reproducing throughout mild winters (Blackman, 1974; Pons, *et al.*, 1995). The occurrence of anholocyclic clones can be the result of environmental change where warmer climates allow for the continuation of asexual reproduction by holocyclic forms (facultative asexual reproduction), or due to genetic change where the aphids are no longer capable of responding to the stimuli which initiate the sexual reproductive process (obligate asexual reproduction) (Moran, 1992). Like *M. persicae* and *R. padi*, anholocyclic clones of *N. ribisnigri* have been identified in Spain and in the South of Britain (Nebreda, *et al.*, 2005; RIS, No date-a).

Aphid polyphenisms and polymorphisms

Aphids occur in a range of different morphs which have specific roles and occur in response to various species-specific and morph-specific environmental cues (Wellings, *et al.*, 1980). It is the aphids' short reproductive generation time which

allows them to ‘monitor’ seasonal changes and adapt quickly to ensure they are utilising their environment effectively.

Known as polyphenisms, these different aphid morphs occur in genetically identical individuals (clones) and display a range of phenotypes varying in morphology, potential fecundity, development time, host plant associations and physiology (Moran, 1992). Evidence also exists for polymorphisms, which are when genetic factors are also involved in determining the phenotype. For example, while the environment stimulates the production of sexual morphs, in the pea aphid *Acyrthosiphon pisum*, whether the male is alate or apterous is determined by a single locus, named *aphicarus* (*api)* (Braendle, *et al.*, 2006). Interestingly, this locus has also been found to be involved in determining the female alate polyphenism (Braendle, *et al.*, 2005).

The polyphenisms exhibited by *N. ribisnigri* include the fundatrix, summer alate and apterous parthenogenetic females, the gynoparae and sexual morphs (including males and oviparae). The induction of alate parthenogenetic females is suggested to be in response to changes in nutrition, host plant, temperature, predation and crowding (tactile stimulation) combined with maternal age, which can be sensed either pre-natally or post-natally depending on the species (Sutherland, 1969; MacKay and Wellington, 1977; Müller, *et al.*, 2001; Hatano, *et al.*, 2010). Some of these factors, such as poor plant nutrition, predation and temperature can, however, cause insect movement and therefore tactile stimulation, so their independence as a factor alone is difficult to determine (Kunert, *et al.*, 2005). In comparison with the alate parthenogenetic aphid, the sexual morphs and gynoparae are usually produced in response to decreasing temperature, short day length and poor nutritional quality, depending on the species (Dixon, 1977).

As well as being adapted to respond to different environmental cues, each morph is also adapted to different environmental conditions, for example the fundatrix is usually the most fecund of all the morphs, being able to exploit the favourable condition on the primary host in the spring, but its morphology differs from the summer apterous parthenogenetic females, by having reduced antennal sensoriation, shorter legs, cauda and siphunculi and a more rounded body (Hille Ris Lambers, 1966). The high reproductive success of the fundatrix demonstrates a trade-off with

the negative cost of reduced mobility, which could impact on survival in adverse conditions (Moran, 1992). The opposite is true of alate morphs, where for wing development to occur, energy is diverted from other physiological processes such as reproduction and development, resulting in the alate form being less fecund and having a longer developmental time compared to that of the apterous form (Zera and Denno, 1997; Finlay and Luck, 2011). Dixon and Wratten (1971) observed that alate *Aphis fabae* had a 30% lower net reproductive rate compared to apterae.

Clonal variation

Throughout the literature, the term clone is used to describe asexual lineages and implies that individuals are genetically identical to their mothers, although Loxdale (2009) indicates that there is little evidence to support this. If there were true clones, one would expect to see no genetic variation between parent and offspring, but intra-clonal variation does exist, expressing itself through for example, the number of offspring and their longevity (Loxdale and Lushai, 2003). Other studies have shown individuals from a single clone of alate *A. fabae* have differing numbers of ovarioles (Dixon, 1987b) while individuals from a clone of *Sitobion avenae* display differences in colour (Alkhedir, *et al.*, 2010).

The development of molecular markers has revolutionised the study of clonal lineages, allowing investigation of genetic variation in addition to the above phenotypic variation. For example, polyphenisms are intra-clonal, inter-morphic differences that occur within a clonal lineage of an aphid, and studies have shown that different morphs of some anholocyclic *S. avenae* and holoyclic *R. padi* clones can be identified by the presence or absence of one or more randomly amplified polymorphic DNA-polymerase chain reaction bands (Lushai, *et al.*, 1997).

Naturally it is not surprising that with the existence of intra-clonal variation, inter-clonal variation also exists. Inter-clonal variation refers to the differences seen between clones and this can be seen through differences in body colour, propensity to become alate, competitive ability, off-plant survival and the number of offspring produced, in response to the same environmental cues, as seen in *A. pisum* (Wolfgang and Braendle, 2001; Hazell, *et al.*, 2005;). Variation has also been identified in *A. pisum* in their levels of defence to natural enemies (Ferrari, *et al.*,

2001; Gwynn, *et al.*, 2005). At a genetic level, DNA fingerprints obtained from several clones of *S. avenae* provided evidence for inter-clonal differences (Carvalho, *et al.*, 1991).

Various hypotheses have been suggested to explain the variation seen within and between asexual lineages, with the earliest proposed by Cognetti, who rejected the hypothesis that parthenogenesis in aphids was a meiotic process and instead proposed a method of endomeiosis, which provides genetic variability to offspring through the formation of homologous chromosomes and chromosomal crossing (Cognetti, 1961). However, on-going research has shown that in the short term, no major genetic rearrangements occur in aphid clonal lineages and they are stable, therefore making endomeiosis unlikely (Blackman, 1971; Carvalho, *et al.*, 1991).

Researchers have now determined that the most likely cause for the observed variation is through mutations, as even a conservative mutation rate, combined with high reproductive rates, overlapping generations and short generation times, can result in thousands of mutations per generation (Loxdale, 2008). The use of molecular techniques has confirmed the occurrence of these mutations and their somatic and germ line origin, meaning that some of these mutations could be adaptive (Lushai, *et al.*, 1998).

Genotype by environment interactions have also been proposed to be responsible for some of the observed variation in certain traits. For example in the pea aphid, a study determined that at the *api* locus, female parthenogenetic clones with different *api* genotypes displayed different propensities to become winged, in response to different environmental cues (Braendle, *et al.*, 2005).

Understanding the intra-clonal and inter-clonal genetic variation which occurs in aphid clonal lineages is important for understanding the diversity of aphids. It is also important to have measures of the extremes of this variation so that it can be accounted for in forecasting (i.e. variation in development) and predator/prey models (i.e. variations in response to predators) to improve their accuracy.

Dispersal and distribution

According to the CABI distribution maps of plant pests (CABI/EPPO, 2003), *N. ribisnigri* is most commonly found throughout Europe and has also been identified in North America, South America and Oceania. Additions to this distribution have since been made, including the Middle East and Central Asia (Blackman and Eastop, 1984; Blackman and Eastop, 2006b).

Collectively aphids display a wide distribution and for the majority of described species this distribution is localised to the temperate regions, where low floral diversity is thought to provide highly abundant host plants which can support aphid species and their large populations (Dixon, 1987c). Their wide distribution can also be attributed to the alate morphs and their ability to migrate long distances (hundreds of kilometres) using air movement, with average flight speeds from 0.8 to 3.3km per hour (Taylor, 1977; Robert, 1987). Accidental introductions have also occurred in some locations such as the introduction of *N. ribisnigri* to California, and its introduction and dispersal throughout New Zealand through the transportation of infested lettuce and seedlings for transplanting (Stufkens and Teulon, 2003; Bugg, *et al.*, 2008).

When considering migratory *N. ribisnigri*, there are two kinds, those that migrate to and from their primary and secondary host plants, and those that are produced throughout the summer in response to certain stimuli, as discussed previously. The most economically important of these is the summer alate which can be separated into non-migratory and migratory morphs, which carry out either trivial low level flights locally (often not taking flight) or undergo significant journeys to colonise new locations respectively (Kring, 1972). It is the migratory aphids which are the most important, due to their potential to distribute plant viruses (not significant for *N. ribisnigri*) and found new colonies on aphid-free crops. When considering the factors which cause high mortality during aphid migration, it is surprising that aphids are considered as successful colonisers.

Colonising a suitable host plant is challenging for alates, as many aphid species exhibit host plant specificity and alates can only control their flight direction and speed in low winds, which can extend the searching time considerably, resulting in desiccation (Powell, *et al.*, 2006). Furthermore, aphid take-off and continued flight

is restricted by temperature, for example when observing the take-off of several aphid species, Dry and Taylor (1970) observed no flight below 14°C, with the majority requiring 20-30°C. However, temperature thresholds for continued flight have been determined to be lower and are essentially the temperatures which allow the continuation of wing movement (Taylor, 1963). It is likely that the limiting factors described above also contribute to the restricted localisation of the majority of aphid species to the temperate regions.

During the summer, dispersal can also occur on a smaller scale through inter-plant movement which can be carried out by non-migratory and apterous aphids. Apterous *Macrosiphum euphorbiae* have been reported to reach speeds of 35 cm per minute (Phelan, *et al.*, 1976). When compared with alates, in the glasshouse, apterous *N. ribisnigri* have been reported to have a higher ability to spread to neighbouring plants during the first week of infestation compared to alates (Diaz, *et al.*, 2012). While, generally, apterous movement is considered to be limited compared to the alate, these studies suggest both morphs can provide effective long and short distance dispersal.

Following dispersal, aphids can exhibit a particular distribution which can be important when considering virus transmission, natural enemies and their control. When considering the in-field distribution of *N. ribisnigri*, it has been reported to infest host plants in a scattered manner, but initially colonising plants near field margins (Mackenzie and Vernon, 1988). Investigations into the distribution of *N. ribisnigri* on iceberg lettuce confirmed that when populations were small they preferred to be within the developing heads, and as populations increased they moved towards the outer leaves (Liu, 2004).

Understanding the dispersal patterns of aphids can help to identify the factors which determine their distribution, which is important information for IPM strategies. Studies on the spatial dynamics of *M. persicae* have indicated that summer migration shows a consistent pattern each year, where the distribution of alates is determined by the location of the main host plants (Taylor, 1977). Incorporation of new technologies, such as molecular markers into these studies can provide value-added information. For example, discrimination of clones at a genetic level can help to monitor their geographical locations and movements which can be vital when

monitoring insecticide resistant clones, as shown for *M. persicae* (Malloch, *et al.*, 2006).

Aphid monitoring and population dynamics

Developing effective methods to monitor aphid populations is important as it can provide information on seasonal variation and identify periods that regularly display high infestations, which when combined with other variables, can be used to optimise decision making on what, where and when control measures should be applied (Harrington, *et al.*, 2007).

To monitor aphids, crop and aerial sampling methods are implemented to capture information about numbers of both apterae and alates. Crop sampling methods include *in situ* counts, destructive sampling, vacuuming and beating, while aerial sampling involves the use of water traps, sticky traps, pheromones and suction traps (Harrington, *et al.*, 2007). Much research has been carried out to improve these sampling methods for aphids on different crops to provide accurate measures of field infestation levels, including the effect of trap colour, shape and spatial positioning (Broadbent, 1948a; 1948b; Dewar, *et al.*, 1982; Trumble, 1982; Idris, *et al.*, 2002; Klueken, *et al.*, 2008;).

One of the most important uses of crop sampling is when it is combined with economic action thresholds, which are developed by quantifying the relationship between aphid densities and yield loss, to estimate the minimum number of aphids required before control measures should be applied (Ragsdale, *et al.*, 2007). This kind of sampling is usually carried out in the field by growers and therefore it is important to remember that, for them to be effective, the sampling techniques developed by researchers need to be altered for use by growers, so that they are simple to use, low cost and the output is easily interpretable (Dent, 2000).

One of the most effective aerial sampling methods providing data on aphid abundance, species distribution and annual flight patterns is provided by a series of 12.2 m suction traps operated by The Rothamsted Insect Survey (Woiwod, *et al.*, 1984). This network has provided vital information, such as the relationship of earlier spring migration of aphids, particularly for anholocyclic clones, with higher winter temperatures, and how these earlier spring migrations by aphids result in

larger spring and early summer populations (Harrington and Woiwod, 2007). Data from this network have been used to provide indicative warnings on likely spring migration to crops and numbers that will be present in the traps until late June (HDC, No date; RIS, No date-b). Unfortunately, *N. ribisnigri* is described as being 'trap shy' as very few aerial *N. ribisnigri* are caught by the suction trap network or in water traps (Collier, *et al.*, 1999).

Current control strategies

Historically, insecticides have been used to control aphid infestations, but due to increasing concerns about insecticide safety and insecticide resistance there is now a demand for alternative control methods and/or safer chemicals (Dewar, 2007). The Pesticide Usage Survey Report for 2007and 2011 for crops including lettuce, endive, radicchio, and Chinese cabbage, indicates that there are, on average, three insecticide applications per crop. In 2007, 71% of these applications were made for the control of aphids, while in 2011 only 34% were made (Garthwaite, *et al.*, 2007; 2011). Since 2007, total insecticide applications over all vegetable crops have reduced, with 12,653 less hectares being treated by 2011 (Garthwaite, *et al.*, 2011).

For *N. ribisnigri* particularly, its specific behaviour of developing in the centre of lettuce heads makes its control with insecticides challenging, as the aphids are often protected from foliar insecticide sprays (Parker, *et al.*, 2002). Furthermore, varying levels of insecticide resistance have been identified in *N. ribisnigri*, to pirimicarb, pyrethroids and organophosphates (Rufingier, *et al.*, 1999; Barber, *et al.*, 1999; 2002; Kift, *et al.*, 2004). Currently, two insecticides are particularly effective against *N. ribisnigri*, the first being a systemic seed treatment using imidacloprid, which is effective for the first few weeks of a crop's life (PSD, 2003). The second is a newer insecticide called spirotetramat, which is also systemic and provides a new mode of action with no cross-resistance to other insecticides at this time (Brück, *et al.*, 2009).

Until recently, the most effective control method was the use of resistant cultivars of lettuce which provided absolute control against susceptible *N. ribisnigri* biotypes, while being safe for humans and the environment. These are cultivated widely, but the selection pressure induced by these monocultures has now resulted in a new resistance-breaking biotype of *N. ribisnigri* (van der Arend, 2003; Smilde, *et al.*,

2009). Therefore, the development of new resistant cultivars with a new mechanism of resistance is required urgently.

Currently, augmentative and classical biological control is not considered a suitable control method for *N. ribisnigri* in the field in the UK. This is because targeting control of *N. ribisnigri* with biological control agents is challenging, as the life-cycles of the natural enemies need to be in synchrony with the pest and crop life-cycle, establishing control before the lettuce heads develop around the aphids, where they then become protected (Bugg, *et al.*, 2008). Furthermore, growers rely on insecticides to control other pests and the use of biological control agents would not complement this (Smith, *et al.*, 2008). For glasshouse crops of Romaine lettuce grown in California, control has been achieved through the use of parasitioids (Smith, *et al.*, 2008), but until recently the absence of a known parasitoid in the UK has been a problem. However, investigations into a parasitoid relatively new to the UK, *Aphidius hieraciorum* (Stary), has shown that it has the potential to be a successful biological control agent for *N. ribisnigri* in the glasshouse (DEFRA, 2005).

The only biological control option available for use in the field is through conservation biological control, which aims to enhance natural enemy numbers through habitat management (Dedryver, *et al.*, 2010). It is recognised that syrphid larvae are some of the most effective predators of aphids and they have been found to be important in regulating aphid populations in the field and laboratory, particularly for organic production (Chambers, *et al.*, 1983; Hopper, *et al.*, 2011). Growers are therefore encouraged to increase their natural enemy numbers around agricultural fields to assist aphid control. Various studies have indicated that establishing flower strips and field margins are good methods to increase natural enemy numbers, fecundity and longevity (Wäckers, 2001; Winkler, *et al.*, 2006; Bianchi and Wäckers, 2008). A more recent study has suggested that the presence of wildflower strips resulted in lower numbers of lettuce aphids on adjacent crops, but this effect decreased with distance, where beyond 10 m there was little effect (Skirvin, *et al.*, 2011). *Alyssum maritimum* (sweet alyssum) has also been shown to improve the biological control of *N. ribisnigri* in organic field grown lettuce in California (Gillespie, *et al.*, 2011). These studies highlight the need for on-going

research to determine where these flower strips should be planted and in what quantity.

Cultural management practices are also available against aphids and in lettuce this includes the use of row covers to prevent aphid introductions during the vulnerable stage of crop development (Rekika, *et al.*, 2009) and the removal of crop residues, which could act as a refuge, following harvest (McDougall, *et al.*, 2004). Post-harvest control methods are also being investigated, including the use of ultra-low oxygen treatment for the export of lettuce to countries where *N. ribisnigri* is a quarantine pest (Liu, 2005).

While various control measures exist for *N. ribisnigri*, establishing the most effective IPM strategy requires a thorough understanding of the biology and behaviour of the pest. For *N. ribisnigri* this information is limited, and further information would support the development of new, and refinement of existing, control measures. This is particularly with regard to supporting decision making by growers on the most effective timing of their treatment applications.

Project aims

The overall aim of this project is to quantify aspects of the life-cycle of both wild-type and resistance-breaking *N. ribisnigri* to inform the development of a more effective and targeted control strategy. The specific objectives are to:

1) Investigate the effects of photoperiod and temperature on the development of parthenogenetic summer aphids.
2) Investigate the conditions required to stimulate development of sexual morphs, egg production, termination of egg diapause and egg hatching.
3) Investigate alternative host plants (to lettuce) and confirm whether *N. ribisnigri* can use them as overwintering hosts.
4) Investigate the population dynamics of *N. ribisnigri* in response to natural enemies and entomopathogenic fungi.
5) Investigate the potential of *Lactuca* species and their relatives to provide new sources of resistance genes which could be used to develop resistant cultivars with new mechanisms of resistance.

Chapter 2: General techniques

This chapter describes the general techniques and aphid rearing methods used in this research. All of the experimental work was undertaken at Warwick Crop Centre, Wellesbourne, which is part of the School of Life Sciences in the University of Warwick.

Aphid biotypes

Four clones and two populations of either wild-type (WT) or host plant-resistance-breaking (abbreviated as Rb in this study) *N. ribisnigri* biotypes were used throughout this research (Table 2.1).

Table 2.1 Aphid biotype name, lineage and known history.

Biotype name	Lineage	History
WT4850a	WT Clone	Collected in September 2003 from a lettuce field in Lincolnshire. Field spray history includes Dovetail® (lambda-cyhalothrin and pirimicarb), Plenum® (pymetrozine), Aphox® (pirimicarb), Nico soap® (nicotine sulphate) and Toppel® (cypermethrin). Clonal line established in the laboratory from a single founding mother.
WTKent10Pop	WT Population	Received on 12 November 2010 from an infested lettuce field in Kent. Maintained as a population.
RbKentPop	Rb Population	Received on 16 October 2009 from an infested lettuce field in Kent on resistant (Nr-gene) cultivars. Maintained as a population.
RbKent	Rb Clone	A clonal line established in the laboratory from a single founding mother taken from RbKentPop. Insecticide screening carried out by Rothamsted Research found no resistance to imidacloprid,

		pirimicarb, lambda-cyhalothrin and pymetrozine when compared with a susceptible standard clone (S. Foster, Personal Communication).
RbUK631	Rb Clone	Received on 3 December 2009. UK geographical location unknown. Clonal line established in the laboratory from a single founding mother.

Insect rearing

Each of the aphid biotypes described in Table 2.1 were reared as continuous cultures to ensure a regular supply of *N. ribisnigri* of all life stages. The WT biotypes were kept in controlled environment (CE) Room 3 (20°C 16L:8D, light:dark) in the Insect Rearing Unit (IRU) at Warwick Crop Centre, while the Rb biotypes were kept in CE Room 6 (16L:8D, 20°C) to reduce any potential cross contamination.

Nasonovia ribisnigri biotype WT4850a was the most utilised culture and therefore a large culture was maintained in Perspex cages with a Velcro mesh front (See Figure 2.2a). Aphid biotypes WTKent10 and RbKentPop were also maintained in Perspex cages as the infested lettuce originally received was transferred into cages to allow a large population to develop to maintain the variation within the population. The remaining biotypes were each cultured on two individual plants which were covered with micro-perforated polypropylene bags (200mm x 500mm; Cryovac®) and kept in Perspex cages (see Figure 2.2b) which reduced potential cross contamination.

Cultures were refreshed every three weeks. Clean plants were provided for the aphid biotypes maintained in Perspex cages. For the remaining biotypes, five aphids from each of the two plants were inoculated, using a fine paint brush, onto two new plants. One of the old plants, the one in the best condition, was also kept as a reserve. WT biotypes were kept on susceptible lettuce plants cv. Saladin (Tozer Seeds Ltd) or cv. Pinokio (Enza Zaden Ltd) and the Rb biotypes were kept on a combination of resistant (Nr-gene) lettuce plants cv. Rotary and cv. Eluarde (Elsoms Seeds Ltd) which provided a consistent selection pressure for the biotypes to maintain their resistance-breaking phenotype.

a)  **b)** 

Figure 2.2 a-b **a)** Caged cultures **b)** Cultures in micro-perforated polypropylene bags.

The following methods of good practice were implemented to avoid cross contamination of the aphid biotypes:

- WT aphid biotypes were always handled first when starting experiments or refreshing the cultures. In this way, should cross contamination occur from the WT biotypes to the Rb biotype cultures, the WT biotype would not be able to survive on the resistant cultivars. The WT biotypes were also regularly tested for cross contamination by screening for colonisation of cv. Rotary or cv. Eluarde.
- A one hour interval was left between handling different aphid biotypes.

Lettuce cultivars (*Latuca sativa*)

Three main lettuce cultivars were used in the experiments. An Iceberg type cv. Saladin was used as the susceptible cultivar (Figure 2.3). To confirm its susceptibility, a preliminary experiment was carried on 15 February 2010 to determine whether Saladin could support the development of WT (4850a) and Rb (RbKentPop) biotypes. Five of each aphid biotype were inoculated onto 8 Saladin plants (40 aphids in total) and placed at 20°C, 16L:8D (CE Room 9). The percentage surviving to adult was determined to be 70% for the WT biotype and 85% for the Rb biotype, indicating that cv. Saladin can support both biotypes.

Two cultivars resistant to WT *N. ribisnigri* were also used (Figure 2.3). Eluarde was a red Oakleaf with *Bremia lactucae* (BL) and Nr-gene resistance, while Rotary was an outdoor Butterhead with BL and Nr-gene resistance (Elsoms, 2011).

Figure 2.3 Cultivars Rotary, Eluarde and Saladin (left to right).

Plant rearing

All lettuce cultivars used in this research were sown in vermiculite and left for one week in CE Room 4 (18°C 16L:8D) to germinate. Seedlings were then transplanted into 400ml square plant pots containing Levington© Seed and Modular Plus Sand compost and left to continue their development in CE Room 4 which was an insect-free room. All cultures and plants were watered bi-weekly.

Blackcurrant plants (*Ribes nigrum*)

On 28 October 2010, blackcurrant cuttings (cv. Ben Alder, supplied by Welsh Fruit Stocks, Hereford) were taken from six year old established blackcurrants at Warwick Crop Centre, Wellesbourne in a field known as Long Meadow Centre (National Grid reference SP 27146 56846). Forty five cuttings of approximately 25cm were taken from healthy ripened stems from just below the bud. These were then planted into the soil of an old Dutch light at Warwick Crop Centre, Wellesbourne (GardenAction, 2010). On 1 November 2011, all the cuttings were transplanted into pots. Prior to this transplanting date, cuttings had been transplanted into pots when required.

Plant disposal

Following each experiment, plants were placed into paper bags and put in a freezer for at least one week before being composted. Plants used in the field trials were also destroyed and composted.

Data recording and statistics

All data recording took place in the IRU main laboratory unless otherwise specified. All data were analysed using Genstat for Windows 14th edition (VSN international Ltd.) and Microsoft Excel 2010. Interpretations of analyses were made using 95% confidence intervals.

Chapter 3: The effect of temperature and photoperiod on the development of *N. ribisnigri*

Introduction

Generally, insects are considered to be ectothermic, meaning that their life history is closely determined by the environmental ambient temperature, where the control of their body temperature depends on the balance between their heat gain and heat loss (Casey, 1992). However, some species have been observed to exhibit some form of thermoregulation through changes in behaviour. For example, many flying insects such as moths vibrate their wings to warm their muscles, which enables flight (Krogh and Zeuthen, 1941), while bees crowd together in the brood area and vibrate their thoracic muscles to maintain a hive temperature of 33-36°C (Kleinhenz, *et al.*, 2003).

Temperature is the most significant factor controlling insect development, particularly due to the effects that it has on enzymes (Damos and Savopoulou-Soultani, 2012). Enzymes are temperature-dependent biological catalysts which enable the metabolism of substrates to occur, providing energy for insect cell growth, development and reproduction (Chown and Nicolson, 2004). Changes in temperature can alter enzymes by changing their conformation (affecting availability of the enzyme), substrate binding (affecting availability of the substrate) and influencing the rate of the reactions they catalyse (Higley, *et al.*, 1986; Neven, 2000). In response to increasing temperatures, enzyme activity increases up until an optimum temperature, beyond which, activity decreases and the enzymes begin to denature (Stoker, 2009). At lower temperatures metabolism slows and the insect becomes inactive (Mellanby, 1939). Various studies have described the specific temperatures at which these changes in growth, development and reproduction occur in numerous insects of economic significance, including aphids, as this information can be used to predict insect presence in the field (Awmack and Leather, 2007; Damos and Savopoulou-Soultani, 2012).

Aphids demonstrate a sigmoidal relationship between development rate and temperature, where within an optimum temperature range, development increases with increasing temperature until an upper temperature is reached, where it then

begins to slow (Dixon and Hopkins, 2010). Monitoring insect oxygen consumption as a measure of metabolic rate demonstrates this, as respiration increases with increasing temperature, until the upper developmental threshold (UDT) is reached (Lamb, 1961; Neven, 2000). Similarly, at low temperatures a lower developmental threshold (LDT) exists where development also begins to slow (Dixon and Hopkins, 2010).

As temperatures exceed these thresholds an insect's survival depends on their physiological adaptations such as regulation of water loss and the suspension, or slowing, of metabolic reactions (Abdullah, 1961). As temperatures continue to increase or decrease, lethal temperatures are reached where insects are described to undergo stupor, prolonged coma, irreversible trauma, followed by death (Chown and Nicolson, 2004). Therefore, depending on an insect's adaptability, temperature can significantly determine its distribution and survival (Mellanby, 1939).

Various methods have been used to record insect growth and development which provide indicators of changes in performance in response to varying temperatures and other environmental factors under which they develop. By using measurements of aphid weight over a period of time, mean relative growth rates (MRGR) and relative growth rates (RGR) can be calculated, which are often used to evaluate aphid performance, as strong correlations between fecundity and growth rates have been observed for some aphid species (Dixon and Wratten, 1971; Lowe, 1974; Leather and Dixon, 1984). However, this relationship is not always observed and other factors, particularly host plant quality, can significantly influence aphid size (Awmack and Leather, 2002; Gwynn, *et al.*, 2005)

Recording developmental time (*D*) is also a common approach, where the time between selected developmental stages is recorded (i.e. from birth to final adult moult) and often expressed as a rate, by using the reciprocal (1/D) (Awmack and Leather, 2007). Developmental time can then be used to calculate the intrinsic rates of increase (r_m), as an estimate of population growth, by relating fecundity to developmental time, rather than undergoing labour-intensive field counts (Awmack and Leather, 2007): $r_m = (\ln Md \times 0.738) / D$, where ln is the natural logarithm, *D* is the pre-reproductive time (nymph to final adult moult) in days and *Md* is the reproductive output of an individual aphid following the adult moult for a number of

days equal to *D*. However, it is important to remember that when using an r_m calculated in the laboratory, a stable age distribution within the population is assumed, which is rarely observed in field populations (Dean, 1974).

The effects of temperature on development have been described for various aphid species including *Rhopalosiphum maidis, Schizaphis graminum, Aphis gossypii* and *M. persicae* (Kuo, *et al.*, 2006; Satar, *et al.*, 2008; Tofangsazi, *et al.*, 2010). Comparisons of the temperature responses of different aphid species under similar climatic rearing regimes have identified variation in their development times, optimum ranges, thresholds and reproductive parameters (Campbell, *et al.*, 1974; Dean, 1974; de Conti, *et al.*, 2010). When clones from the pea aphid, *A. pisum* were compared, there was a larger variation in development parameters between clones from the same population, than among populations (Lamb, *et al.*, 1987). Similarly, significant differences in development time and fecundity have been recorded between four biotypes of *A. pisum* collected from four geographically-defined populations (Kilian and Nielson, 1971). It has been suggested that these observed differences, within and between species, could be the result of adaptation to geographic variations in temperature.

Campbell *et al.* (1974) found that the development times of *A. pisum* and *Brevicoryne brassicae,* when kept at 10°C, were longer for individuals collected from warmer climates, indicating their temperature requirements may have been adapted to local rearing regimes. Similarly, the LDT for *A. pisum* populations collected from different latitudes across North America decreased as the climate became cooler (Hutchison and Hogg, 1984). However, other studies have shown no evidence that variation in development rates, developmental thresholds and aphid weight is related to adaptation to the climate at different latitudes from which the aphids were collected (Lamb, *et al.*, 1987; Lamb and Mackay, 1988). As a result of this ambiguity, caution should be used when using temperature parameters for aphid species collected from one geographic location, to predict the activity of the same aphid species collected elsewhere.

Determining the temperature requirements and development parameters for insects is usually achieved by measuring development time in the laboratory at various constant temperatures, such as every 2°C, over a 20°C range (Collier and Finch,

1985). However, the accuracy of using laboratory-collected data to represent field populations is considered to be limiting as the optimum rearing regimes used usually provide minimum development times (Higley, *et al.*, 1986). Furthermore, laboratory experiments cannot include in their predictions, the influence of other factors only found in the field environment such as variable weather, predation and plant-aphid interactions (Bommarco and Ekbom, 1996). Estimations of the development rates of *Metapolophium dirhodum* obtained from field observations showed that while they developed more slowly at lower temperatures, the estimated lower development thresholds were similar to those determined in the laboratory at constant temperatures (Cannon, 1984). For *A. pisum,* apterae and alates exposed to constant temperatures in the laboratory and fluctuating temperatures in the field had similar development times for the mean of the first three instar periods (Hutchison and Hogg, 1984). Similarly, apterous *A. pisum* kept under both constant and field temperatures provided a similar value for the average day-degree requirement for complete development from nymphs to adult (Campbell and Mackauer, 1977). A study on *B. brassicae,* however, found the mean temperature for the maximum rate of population increase was lower in the field, at 16.7°C, than in laboratory experiments at 22°C (Lamb, 1961).

In the laboratory, fluctuating temperatures (providing daily means) are often used to simulate field rearing regimes, which are then compared to constant temperature data. For the sowthistle aphid, *Hyperomyzus lactucae,* development rates, life span and age-specific survival, reproduction and temperature coefficients did not vary between constant and fluctuating temperatures, but fluctuating temperatures did affect their lethal high and low temperature limits which varied with the pattern and amplitude of the fluctuations (Shu-sheng and Hughes, 1987). Campbell *et al.* (1974) also recorded no differences in development rate between constant and fluctuating temperatures. However, a study on *M. persicae* found that fluctuating temperatures resulted in higher UDT and optimal temperatures, combined with improved survival, compared to recordings at constant temperatures (Davis, *et al.*, 2006). Collectively, these observations indicate that caution should be employed when using constant temperature data, as they could underestimate thresholds and development at higher temperatures, meaning that temperature-based forecasts could be unreliable.

Generally, aphids develop through four nymphal instars, undergoing ecdysis at the end of each stage, before reaching their adult form, although five nymphal instars have been recorded for some aphid species (Rohitha and Penman, 1983). For development from one stage to the next, insects require specific amounts of 'heat accumulation', which remain constant for that species. This thermal requirement over time is referred to as physiological time and can be expressed in day-degree units (D°), where one day-degree is accumulated for each degree that the average temperature remains above the LDT for a 24 hour period (Herms, 2004). Therefore, insects require a specific number of day-degrees (a thermal constant) to complete each developmental stage.

Determining the number of day-degrees allows researchers to make estimates of when development will be completed in a field situation by determining the accumulation of day-degrees from a set 'biofix' date (UCIPM, 2003). In the UK, the biofix date used is often 1 February, which is when temperatures begin to rise in the spring and insects are ready to begin post-diapause development (Collier and Finch, 2001). The following equation is the accepted method for calculating day-degrees (Herms, 2004):

$$\text{Day-degrees} = (\text{max temperature} + \text{min temperature})/2 - \text{LDT}$$

Various adaptations of this method exist which aim to improve its accuracy. These include the sine and triangle methods where the effects of the UDT on day-degree accumulations are considered in addition to the LDT (UCIPM, 2003). On reaching the UDT, various 'cut-off' methods can be implemented where one can assume; a) no more development occurs (vertical cut-off); b) development continues slowly (intermediate cut-off) or c) development occurs at a constant rate (horizontal cut-off) (Baskerville and Emin, 1969; UCIPM, 2003). More recent refinement, as a result of the increasing numbers of growers using their own on-site meteorological stations, include calculating separately, morning and afternoon day-degree estimates and using hourly temperature data to improve accuracy (Roltsch, *et al.*, 1999).

Day-degree models have often been used successfully in predicting insect activity, as temperature is generally the main determinant of growth. These include models for

Delia radicum and *Plutella xylostella* (Butts and McEwen, 1981; Collier and Finch, 1985; Eckenrode and Chapman, 1972). In the UK, the thermal requirements for *N. ribisnigri* are unknown, but a day-degree model, using the LDT of *Pemphigus bursarious* (4.4°C) as an estimate, has been evaluated, which showed that the dates when the first *N. ribisnigri* was captured and the dates when peak numbers were found on plants, were strongly correlated with the accumulated day-degrees from 1 February each year (Collier and Harrington, 2001).

While day-degree forecasts have been made for a number of pests, there are various factors which limit the accuracy of their predictions. For example, they do not take into consideration the variation between individuals in a population, and they also assume that the relationship between development rate and temperature is linear (Finch, *et al.*, 1996). Many stochastic mathematical models have been developed to improve accuracy of these forecasts, such as the Monte Carlo Simulation model for cabbage root fly (*D. radicum*), carrot fly (*Psila rosae)* and bronzed blossom beetle (*Meligethes* spp.), which accommodates variability within insect populations, resting phases (diapause and aestivation) and the passage through different development stages (Phelps, *et al.*, 1993; Collier and Finch, 2001). Such models, however, do not consider other non-temperature variables influencing development, particularly photoperiod and host plant nutrition (McMaster and Wilhelm, 1997).

The effects of photoperiod on aphid development have been studied extensively with regard to requirements for the production of sexual forms and egg diapause, yet effects on developmental and reproductive parameters of parthenogenetic aphid forms have been overlooked. Should photoperiod have an influence on development time, this could impact the reliability of all temperature-based forecasts. This has been observed for other pest insects where for example, the development time of the fifth larval instar of the black lyre leafroller (*Cnephasia jactatana*) was similar at 12L:12D and 6L:18D, but lengthened under complete light, complete dark and 18L:6D (Ochieng-Odero, 1991). Higley *et al.* (1986) suggested that as genetics and photoperiod are linked with regulating insect hormones, which in turn regulate development through changes in enzyme concentrations, it would therefore not be surprising if photoperiod did have an effect on development.

For *N. ribisnigri*, there is no information on the effects of photoperiod on development, but a study on *H. lactucae,* reported little effect of photoperiod in the range of 12L:12D to 16L:8D on development rates, survival and fecundity when temperature was kept constant (Shu-sheng and Hughes, 1987). However, investigations into *A. gossypii* on *Cucurbita pepo* found that the r_m and finite rates of increase significantly increased with increasing day length, and nymphs developed faster at 18L:6D compared with 12L:12D and 6L:18D when kept at 25°C (Aldyhim and Khalil, 1993).

While it is unclear whether photoperiod has its own 'separate' effect on development, it has been shown to be involved in complex interactions with other factors such as light intensity and temperature, which is likely to be the cause of the difficulty in isolating any 'separate' effects. A study by Wyatt and Brown (1977) compared the development of four species of aphids (all greenhouse pests) in response to different day lengths, light intensities and temperatures and found that the pre-reproductive time was shortest during long warm days, but delayed during short warm days, with high light intensity delaying it further. Sometimes, however, responses were species-specific. For example, *A. gossypii* developed faster during long cool days compared with *M. persicae, Brachycaudus helichrysi* and *Macrosiphoniella sanborni*, whose development slowed. This study not only emphasises the importance of day length but also light intensity as factors affecting development. A recent study confirmed that, independently, high light intensity increased the fresh weight and the number of offspring of ten clones of *S. avenae*, while lower light intensities increased the propensity to become alate (Alkhedir, *et al.*, 2010).

In addition to the abiotic factors discussed, studies also support the effects of biotic factors, particularly host plants, in influencing aphid performance and development, both between and within aphid species. For example, day-degree estimates, and the LDT estimated for *R. maidis* in Taiwan on corn leaf, differ from those estimated in the United States for *R. maidis* on barley leaves. It was suggested that a combination of low temperatures and host plant could be causing the variation (Kuo, *et al.*, 2006). Similarly, at a constant temperature of 10°C *N. ribisnigri* showed longer developmental times, shorter reproductive periods and lower total fecundity on *L.*

sativa cv. Cuatro Estaciones than on cvs. Divina and Criolla Blanca (Vasicek, *et al.*, 2000). In a study on the effect of host plant growth stage and feeding site, *M. persicae* and *A. gossypii* displayed different feeding site preferences and development times. However, both species demonstrated shorter development times on young chrysanthemum plants compared with flowering plants (Guldemond, *et al.*, 1998). *Sitobion avenae* also exhibits higher development rates, MRGR and r_m on the ear of wheat, where the relationship of temperature and development rates is linear, compared with development on flag leaves, where the relationship is non-linear (Acreman and Dixon, 1989).

In the present study, the effects of temperature and photoperiod on the development of WT and Rb *N. ribisnigri* on resistant and susceptible lettuce cultivars were determined, through measurements of development time/rates and estimation of the r_m. Lower developmental thresholds and thermal requirements were also estimated with the aim of producing a more reliable day-degree model. This would provide growers with better predictions of the timing of *N. ribisnigri* colonisation, help them to identify lettuce plantings at risk and indicate when control measures should be applied, all of which are vital for effective control of this pest (Parker, *et al.*, 2002).

Materials and Methods

3.1 Effects of temperature on the development of WT and Rb *N. ribisnigri* reared on susceptible and resistant lettuce cultivars

The effects of five different temperatures (5, 10, 15, 20 and 25°C) on the development of WT and Rb *N. ribisnigri* were determined. For each temperature, the experiment was replicated on three occasions, except at 5°C where there were only two replications due to the long development times required by the aphids. Replications at each temperature were carried out at different times due to the intense monitoring required for each experiment and the limitations imposed by the availability of CE rooms.

The experiment consisted of six treatments. WT and Rb *N. ribisnigri* were reared on three cultivars of lettuce, cvs. Saladin (susceptible), Eluarde (resistant), Rotary (resistant), with each treatment consisting of ten lettuce plants (60 plants per

experiment). WT *N. ribisnigri* reared on cv. Saladin was used as a control. Twenty plants of each cultivar were grown as described in Chapter 2. The plants were approximately 21 days old. This is the stage at which their 4th true leaf had unfolded, which is designated as growth stage 14 when using the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) identification key for leafy vegetables (forming heads) (Feller *et al.,* 1995).

The *N. ribisnigri* biotypes used in the experiment included the WT clone designated WT4850a and the Rb population designated RbKentPop (See Chapter 2 for biotype details). New-born nymphs of each of these biotypes were used in each experiment. These were obtained by releasing 45 alate adults into a cage containing three lettuce plants cv. Saladin, where they were left for 24 hours at 20°C 16L:8D to produce nymphs (repeated individually for Rb and WT biotypes). After 24 hours, 10 plants of each of the lettuce cultivars were inoculated with one new born nymph of the WT *N. ribisnigri* clone and the remaining plants were inoculated with one nymph of the Rb clone, using a fine paintbrush.

The 60 inoculated plants were then covered individually with micro-perforated polypropylene bags (200mm x 500mm; Cryovac®), which were secured with an elastic band. At 10°C (CE Room 5) and 20°C (CE Room 6) the experimental design was arranged over two shelves (Figure 3.1.1b) while at 15° (CE Room 9) and 25°C (CE Room 10) the design was arranged over a single shelf (Figure 3.1.1c). Plants were arranged in a randomised block design with ten blocks over four rows and 15 columns. The CE rooms could not operate at 5°C, so a Sanyo (MLR-351) plant growth chamber was used and the plants were arranged over five shelves (two blocks per shelf) (Figure 3.1.1a). The photoperiod was kept at 16L: 8D in all replications. Relative humidity ranged between 67-75% at 5°C, 84-93% at 10°C, 66-93% at 15°C, 84-94% at 20°C and 53-73% at 25°C.

Collecting the data at 5°C was challenging and required several restarts as the incubator malfunctioned due to operating at the limits of its temperature range. Tiny Tags© were used to record the temperature in each CE room and were placed in the centre of each shelf, where readings were taken every 30 minutes

a)  **b)** 

c) 

Figure 3.1.1 a-c **a)** Sanyo (MLR-351) plant growth chamber used to rear aphids at 5°C **b)** Large CE room used at 10 and 20°C **c)** Small CE room used at 15 and 25°C.

The following data were collected for the individual aphids in each treatment:

- *Development time to adult* - The number of days it took each individual aphid to reach adulthood was recorded. Aphids were checked from day 29, 13, 8, 5 and 4 onwards at 5, 10, 15, 20 and 25°C respectively, until all the surviving aphids had reached adulthood. This schedule was developed using previously-determined development times as a guide (Diaz and Fereres, 2005).
- *Adult morph* - whether the adults were apterous or alate.
- *Mortality* - the number of nymphs which died before reaching adulthood.
- *Intrinsic rate of increase* - $r_m = (\ln Md \times 0.738) / D$ (Wyatt and White, 1977).

- *Achieved fecundity* - the total number of nymphs produced by each aphid was recorded until death. The nymphs were counted and removed to ensure only the original adult aphid remained on the plant.

When making data recordings, any plants where aphids were found to be missing after two consecutive recordings, or were dead, were removed from the experiment. Plants were also changed during each experiment, as they grew to an unmanageable size and were replaced with approximately 21 day old plants. This took place approximately every two weeks but the period was increased to six weeks at 5°C due to slower plant development. Measurements of growth rates were attempted but the microbalances available could not accurately weigh individual aphids, which can weigh as little as 30µg (Dixon, 1998). Table 3.1.2 shows the dates each experiment was started.

Table 3.1.2 Start dates of each temperature experiment.

Temperature (°C)	Replication	Date
5	1	24 September 2010
	2	29 January 2011
10	1	17 March 2010
	2	3 September 2010
	3	5 April 2011
15	1	28 May 2010
	2	29 July 2010
	3	8 October 2010
20	1	7 July 2010
	2	17 September 2010
	3	26 October 2010
25	1	19 March 2010
	2	28 May 2010
	3	11 August 2010

3.2 Effects of temperature on WT *N. ribisnigri*

Due to the relatively low number of replicates and high numbers of treatments present in Experiment 3.1, an experiment was designed to determine the effects of exposure to 10, 15, 20 and 25°C on 60 individual aphids of the control treatment only (WT + Saladin). This larger data set could then be used to determine the LDT and thermal requirements more accurately. The 5°C treatment was excluded because of the longer development time required, combined with the time constraints and incubator malfunctions.

The experiment was carried out in the same way as Experiment 3.1 and 60 replicates were used and arranged on a single shelf (4 x 15). A blocking structure was not used as there was only one treatment. Data recorded included development times, r_m and the aphid morph. The experiments were repeated once per temperature and were concluded once the r_m could be estimated. The 25°C experiment began on 19 November 2011, the 20°C experiment on 15 November 2011, the 15°C experiment on 3 February 2012 and the 10°C on 5 January 2012.

3.3 Effects of aphid biotype on the propensity to become alate

Differences in the propensity to become alate were determined at 20°C 16L:8D for WT and Rb *N. ribisnigri* biotypes. The experiment consisted of three treatments. WT *N. ribisnigri* were reared on cv. Saladin and Rb *N. ribisnigri* was reared on cv. Saladin and cv. Rotary, with each treatment consisting of 32 lettuce plants (96 plants per experiment). New born nymphs of WT and Rb *N. ribisnigri* were obtained and plants were grown as per Experiment 3.1. Individual aphids were inoculated onto each lettuce cultivar according to the treatments and covered individually with micro-perforated polypropylene bags (200mm x 500mm; Cryovac®) and secured with an elastic band. Plants were arranged in a randomised block design with 4 blocks over 8 rows and 12 columns on a single shelf in CE Room 6 (20°C 16L:8D). Aphids were then left to develop to adulthood, when their adult morph (alate or apterous) was recorded.

3.4 Effects of photoperiod on the development of different aphid biotypes

The effects of two different photoperiods (14L:10D and 16L:8D) on aphid development were determined for five biotypes of *N. ribisnigri* on cvs. Saladin and Rotary (Table 3.4.1). In addition, the shorter photoperiod was tested at 15 and 20°C, while the longer photoperiod was tested at 15°C to give three rearing regimes. For each rearing regime (photoperiod x temperature combination), three replications were conducted to give nine experiments in total. Each experiment contained 60 plants (6 replicates per treatment) and was carried out at different times due to the intense monitoring required and the availability of the CE rooms.

Table 3.4.1 The 10 treatments (five aphid biotypes on cv. Saladin and cv. Rotary) tested under three rearing regimes (20°C 14L:10D, 15°C 14L:10D and 15°C 16L:8D) to determine the effects of photoperiod on the development of *N. ribisnigri*.

Treatment (aphid biotype and lettuce cultivar)
WT4850a + Saladin
WTKent10Pop + Saladin
RbKent + Saladin
RbUK631 + Saladin
RbKentPop + Saladin
WT4850a + Rotary
WTKent10Pop + Rotary
RbKent + Rotary
RbUK631 + Rotary
RbKentPop + Rotary

Plants were grown as described in Chapter 2 and used at approximately 21 days. This is the stage at which their 4th true leaf had unfolded (BBCH growth stage 14). New born nymphs of each aphid biotype were obtained by releasing 30 alate adults into a cage containing two lettuce plants cv. Saladin (repeated for each aphid biotype). After 24 hours, individual new-born aphids were inoculated onto the test

plants of each lettuce cultivar according to the treatment list and covered individually with micro-perforated polypropylene bags (200mm x 500mm; Cryovac®) which were secured with an elastic band. Plants were arranged in a randomised block design consisting of six blocks over 4 rows and 15 columns on a single shelf in a small CE room (CE Rooms 8 or 9). The lighting levels in CE room 8 (100 watt lamps) ranged between 4060-4710lux and in CE room 9 (100 watt lamps) between 3600-4500lux.

The data collected included development time to adult, adult morph and $r_{m.}$ Missing and dead aphids were treated in the same way as in Experiment 3.1. Once the r_m value could be calculated the experiment was concluded. Table 3.4.2 shows the dates each experiment was started.

Table 3.4.2 Start dates of each photoperiod experiment.

Condition	Replication	Experiment start date
14L:10D 15°C	1	23 February 2012
	2	16 March 2012
	3	18 May 2012
14L:10D 20°C	1 & 2	24 May 2011
	3	15 July 2011
16L:8D 20°C	1	24 February 2011
	2	26 October 2011
	3	15 November 2011

Statistical analysis

3.1 Effects of temperature on the development of WT and Rb *N. ribisnigri* reared on susceptible and resistant lettuce cultivars

Analyses of the development time, r_m and total fecundity at each temperature, and for each treatment, were performed using ANOVA. A log10 data transformation was carried out to normalise the development time data. Achieved fecundity did not

require transformation. The r_m data were not transformed, as there were more observations for the larger fitted values, which resulted in small SED/LSD values which would over-emphasise the differences between treatments with higher means. This also means that when using the untransformed data the LSDs may be too large to detect differences between treatments with relatively low means, so care must be taken.

The treatment factors used in the ANOVA included treatment*temperature. Aphid biotype and lettuce cultivar were combined to form the treatment factor to simplify the data, as there were too many missing values leading to a significant number of missing treatment factor combinations for aphid biotype*lettuce cultivar* temperature, when aphid biotype and lettuce cultivar were used as individual factors. Also, due to the small amount of data available, the WT Eluarde and WT Rotary treatments were not included in the ANOVA, as when estimates of the missing values were made by the statistical software, they were significantly erroneous when compared to the calculated means from the raw data.

The blocking structure used in the ANOVA included Occasion.Temperature which accounted for any variation observed between the development parameters at each temperature within each occasion. Interpretation of the data was made using both treatment means and 5% LSD values. Analyses were carried out combining both apterae and alates and also with the alates excluded. To exclude the alates, the data for them was replaced with missing values as the complete removal of the data resulted in an unbalanced design, which cannot be analysed by ANOVA.

Survival to adulthood was analysed using ANOVA. The number of aphids surviving to adulthood for each treatment at each temperature was determined, and a proportion was calculated out of a total of 30. The treatment factors used in the ANOVA included Temperature*Treatment. The blocking structure used included Occasion.

To analyse the differences in the proportion of alate morphs between treatments, a general linear model was used to model binomial proportions (logistic regression). The treatments WT+Eluarde and WT+Rotary were excluded from the analysis as there were too many missing values. The proportions used were the number of alates

observed out of the total observations for each treatment. The fitted terms of the model used were:
Occasion+Temperature+Occasion.Temperature+Treatment+Temperature.Treatment. Model terms were fitted individually. Predictions from the regression model were used to provide means for the effect of temperature and treatment individually on the proportion of alates produced.

3.2 Effects of temperature on WT *N. ribisnigri*

To determine the LDT, development rates were determined using the reciprocal of development time (1/development time) at each temperature (10, 15, 20 and 25°C) collected for the WT4850a biotype on cv. Saladin in Experiment 3.2. A two-sample bionomial test (two-tailed) was also performed to determine the equality of the proportions of alates between the four temperatures with a 95% confidence interval.

The reciprocals were also determined from the data collected at 5, 10, 15, 20 and 25°C for the WT4850a and RbKent biotypes on cv. Saladin in Experiment 3.1.

Linear regressions were then applied to the three data sets of the reciprocals, where the relationship between mean rate of development (*y* dependent variable) and temperature (*x* independent variable) were described using linear regression (Campbell and Mackauer, 1977):

$$Y = a + bx$$

Where *Y* is the development rate, *a* is the intercept, *b* is the slope of the line and *x* is the temperature.

The LDTs were extrapolated for the three treatments using the x-intercept method (where the value of *x* is determined when *y* equals zero) (Arnold, 1959):

$$x = \frac{-a}{b}$$

Day-degrees for the three treatments were calculated using the formula DD= 1/b (Campbell, *et al.*, 1974; Tofangsazi, *et al.*, 2010).

3.3 Effects of aphid biotype on the propensity to become alate

A two-sample bionomial test (two-tailed) was performed to determine the equality of the proportions of alates between the three treatments with a 95% confidence interval. The control (WT+Saladin) data were compared with Rb + Saladin and Rb + Rotary. Rb + Saladin data were compared with Rb + Rotary.

3.4 Effects of photoperiod on the development of different aphid biotypes

An ANOVA was carried out on the untransformed means for development time to adult and the r_m. The blocking structure used included Occasion/Blocking to account for any variance between occasions and the differences between the blocks within each occasion. The treatment factors included rearing regime*aphid*lettuce. No data were available for WT+Eluarde and WT+Rotary treatments, which were left in the data set as missing values. Analysis of the data was carried out combining both apterae and alates, as little effect was seen in analysing them separately as observed in the analysis of Experiment 3.1. Interpretations of the data were made using both treatment means and 5% LSD values.

Results

3.1 Effects of temperature on the development of WT and Rb *N. ribisnigri* reared on susceptible and resistant lettuce cultivars

Temperature recordings were made during each experiment using Tiny Tags© to determine the deviation from the temperatures at which the rooms/growth chamber were set (5, 10, 15, 20 and 25°C). As significant variation was observed from these set temperatures, the mean temperature recorded during each experiment was used as the treatment temperature to improve the accuracy and estimates of the development data. The revised treatment temperatures were 5.5, 12.5, 15.9, 21.4 and 26.4°C.

Development time

Figure 3.1.3 illustrates the development time to adult at each temperature for each of the six treatments. As expected, development time to adult was greatest at lower

temperatures, while higher temperatures resulted in reduced development times. The highest temperature of 26.4°C produced the shortest mean development time for the control treatment (WT + Saladin) of 6.25 days, followed by 8 days at 21.4°C, 11.48 days at 15.9°C, 16.76 days at 12.5°C and 41.54 days at 5.5°C. Development times for the control treatment and treatments including the Rb *N. ribisnigri* biotype were very similar at each temperature.

Unexpectedly, at the lower temperatures of 5.5, 12.5 and 15.9°C a small number of the WT biotype reared on the resistant cvs. Eluarde and Rotary survived to adulthood, where 100% mortality would have been expected. For these treatments the development time was not as consistent as for the other treatments. It was also observed that at the lower temperature of 5.5 and 12.5°C the aphids became larger and darker in colour compared with 15.9, 21.4 and 26.4 °C, where they were light orange and often smaller.

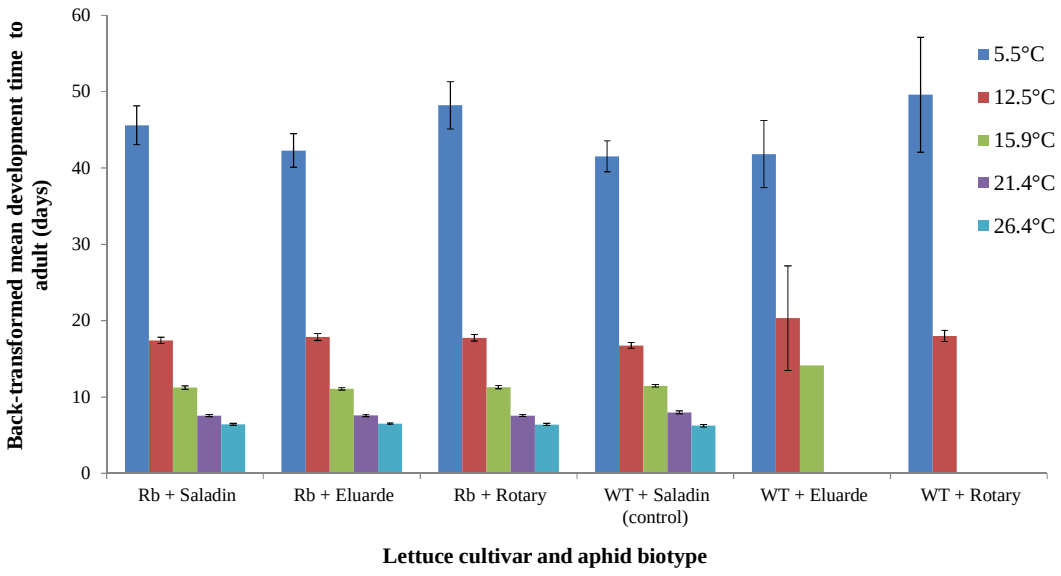


Figure 3.1.3 Development time (days) to adult for each treatment at five constant temperatures (5.5, 12.5, 15.9, 21.4 and 26.4°C) including the standard error (SE).

An ANOVA was performed on the pooled data for apterous and alate aphid morphs (excluding WT + Eluarde and WT + Rotary treatments) and this described a significant overall effect of temperature on development time ($F(4,9)= 937.95$, p

<0.001). There were no individual effects of the treatments and therefore the Rb *N. ribisnigri* biotype displayed similar development times to the WT biotype at each temperature. An interaction between temperature and treatment was observed ($F(12,440)=2.30$, $p=0.008$) as each treatment had significantly different development times at each temperature as demonstrated in Table 3.1.4.

When using the LSDs to make comparisons between treatments at the same temperature, only at 5.5°C were any differences found, indicating there is more variation between treatments in development time at this temperature (Table 3.1.5).

Table 3.1.4 LOG10 transformed (t) and back transformed (bt) mean development times (days) to adult from the ANOVA analysis for each treatment at 5.5, 12.5, 15.9, 21.4 and 26.4°C; n = number of observations; *Tn*= total number of possible observations. Transformed treatment means with different letters in a **column** are significantly different between temperatures.

	Treatment												
	WT Saladin (Control)			**Rb Saladin**			**Rb Eluarde**			**Rb Rotary**			
°C	n	t	bt	n	t	bt	n	t	bt	n	t	bt	*Tn*
5.5	13	1.6144^{a}	41.15	17	1.6481^{a}	44.47	14	1.6182^{a}	41.51	14	1.6734^{a}	47.14	20*
12.5	25	1.2238^{b}	16.74	26	1.2363^{b}	17.23	27	1.2482^{b}	17.71	28	1.2475^{b}	17.68	30^
15.9	25	1.0587^{c}	11.45	30	1.0482^{c}	11.17	30	1.0427^{c}	11.03	29	1.0519^{c}	11.27	30^
21.4	21	0.9025^{d}	7.99	24	0.8796^{d}	7.58	27	0.8783^{d}	7.56	26	08784^{d}	7.58	30^
26.4	12	0.8014^{e}	6.33	29	0.8048^{e}	6.38	27	0.8111^{e}	6.47	25	0.8037^{e}	6.36	30^

* LSD used for comparisons within a treatment between temperatures with minimum and maximum observations e.g. 5.5 and 12.5 (*Tn*20-30) = 0.04070

^ LSD used for comparisons within a treatment between temperatures with maximum observations i.e. 12.5 and 15.9 (*Tn*30-30) = 0.03640

Table 3.1.5 LOG10 transformed (t) and back transformed (bt) mean development times (days) to adult from the ANOVA analysis for each treatment at 5.5, 12.5, 15.9, 21.4 and 26.4°C; n = number of observations; *Tn*= total number of possible observations. Transformed treatment means with different letters in a **row** are significantly different at that temperature.

	Treatment												
	WT Saladin (Control)			**Rb Saladin**			**Rb Eluarde**			**Rb Rotary**			
°C	n	t	bt	n	t	bt	n	t	bt	n	t	bt	*Tn*
5.5	13	1.6144^{a}	41.15	17	1.6481^{bc}	44.47	14	1.6182^{ac}	41.51	14	1.6734^{b}	47.14	20*
12.5	25	1.2238^{a}	16.74	26	1.2363^{a}	17.23	27	1.2482^{a}	17.71	28	1.2475^{a}	17.68	30^
15.9	25	1.0587^{a}	11.45	30	1.0482^{a}	11.17	30	1.0427^{a}	11.03	29	1.0519^{a}	11.27	30^
21.4	21	0.9025^{a}	7.99	24	0.8796^{a}	7.58	27	0.8783^{a}	7.56	26	08784^{a}	7.58	30^
26.4	12	0.8014^{a}	6.33	29	0.8048^{a}	6.38	27	0.8111^{a}	6.47	25	0.8037^{a}	6.36	30^

* LSD used for comparisons between different treatments at the same temperature which have minimum observations e.g. 5.5 (*Tn*20-20) = 0.03142

^ LSD used for comparisons between different treatments at the same temperature which have maximum observations e.g. 12.5 (*Tn*30-30) = 0.02565

When the data were re-analysed after converting the alate data to missing values, the ANOVA produced a similar output, with a significant effect overall of temperature ($F(4,9)=761.50$, $p=0.001$), no effect of treatment and a significant treatment and temperature interaction ($F(12,402)=2.59$, $p=0.003$). This increase in significance for the interaction is due to an increase in the variation between the treatments and a reduction in the between-replicate variation.

Survival

An ANOVA was performed on the proportion of aphids reaching adulthood for each treatment at each temperature. Overall there was a significant effect of temperature on the proportion of aphids reaching adulthood ($F(4,52)=3.40$, $p=0.015$), with the highest proportion surviving to adulthood observed at 15.9°C (Figure 3.1.6). Overall survival to adulthood at 5.5, 21.4 and 26.4°C was significantly lower than at 15.9°C. Generally, survival decreased as temperatures increased and decreased from 15.9°C, suggesting that 15.9°C is the optimum temperature for survival of both *N. ribisnigri* biotypes.

Table 3.1.6 Mean proportion of aphids surviving to adulthood at each temperature (LSD = 0.0995).

°C	Proportion surviving to adulthood
5.5	0.5563ab
12.5	0.6389bc
15.9	0.6667c
21.4	0.5444ab
26.4	0.5167a

A significant effect of treatment was observed ($F(5,52)=98.97$, $p=<0.001$), with all the Rb biotype treatments having a higher proportion of aphids surviving to adulthood compared with the control (Figure 3.1.7). This suggests that the Rb biotype has better survival on all of the cultivars. As previously discussed, at 5.5, 12.5 and 15.9 °C, a small proportion of WT *N. ribisnigri* survived on the resistant cvs. Rotary and Eluarde, where 100% mortality was expected.

Table 3.1.7 Mean proportion of aphids surviving to adulthood for each treatment (LSD = 0.1090).

Treatment	Mean proportion surviving to adulthood
WT + Saladin (control)	0.6796b
Rb + Saladin	0.8929c
Rb + Eluarde	0.8763c
Rb + Rotary	0.8563c
WT + Eluarde	0.1163a
WT + Rotary	0.0863a

Table 3.1.8 illustrates the proportion of aphids surviving to adulthood for each treatment at 5.5, 12.5, 15.9, 21.4 and 26.4°C generated from the ANOVA. A significant interaction between treatment and temperature was observed ($F(20,52)=1.90$, $p=0.033$).

For the control, survival was significantly lower at 26.4°C compared to 12.5, 15.9 and 21.4°C. Survival was also low at 5.5°C suggesting that these temperatures were negatively affecting survival of the WT biotype. For the Rb biotypes low survival such as this was only observed at 5.5°C.

Table 3.1.8 Mean proportion of aphids surviving to adulthood for each treatment at 5.5, 12.5, 15.9, 21.4 and 26.4°C (LSD = 0.2438).

	Treatments					
°C	**WT + Saladin (control)**	**Rb + Saladin**	**Rb + Eluarde**	**Rb + Rotary**	**WT + Eluarde**	**WT + Rotary**
5.5	0.631ef	0.831fgh	0.681fg	0.681fg	0.281acd	0.231abcd
12.5	0.833fgh	0.867fgh	0.900fgh	0.933gh	0.100abc	0.200abcd
15.9	0.833fgh	1.000h	1.000h	0.967gh	0.200abcd	0.000a
21.4	0.700fg	0.800fgh	0.900fgh	0.867fgh	0.000a	0.000a
26.4	0.400de	0.967gh	0.900fgh	0.833fgh	0.000ab	0.000ab

Achieved fecundity

Figure 3.1.9 illustrates the mean achieved fecundity at each temperature for each of the six treatments.

The highest mean achieved fecundity for the control was observed at 12.5°C with a mean of 25.84 nymphs, followed by 19.81 nymphs at 21.4°C, 17.69 nymphs at 5.5°C, 16.25 nymphs at 26.4°C and 15.52 nymphs at 15.9°C. The Rb biotype on the cvs. Saladin and Rotary also had the highest achieved fecundity at 12.5°C with 65.23 and 44.50 nymphs respectively, suggesting that this could be the optimum temperature for reproduction for both WT and Rb biotypes. At 12.5, 15.9 and 21.4°C the control had a much lower mean fecundity than the three Rb biotype treatments. The highest achieved fecundity recorded for a single female was 111 nymphs at 15.9°C by the Rb + Eluarde treatment.

Out of the 26 aphids which survived in the WT + Eluarde and WT + Rotary treatments, eight did not reproduce, 17 produced between one and nine nymphs and one produced 57 nymphs.

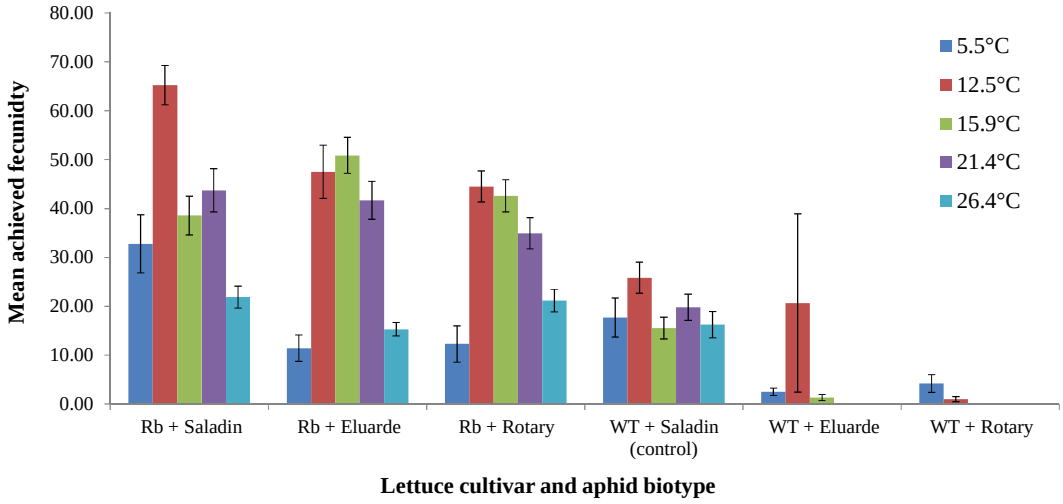


Figure 3.1.9 Mean achieved fecundity at five constant temperatures (5.5, 12.5, 15.9, 21.4 and 26.4°C) for each treatment including the SE.

An ANOVA was performed on the pooled data for apterous and alate aphid morphs (excluding WT + Eluarde and WT + Rotary treatments) and this described a significant overall effect of temperature on fecundity ($F(4,9)=11.13$, $p=0.002$).

There was also a significant effect of treatment ($F(3,440)=43.14$, $p<0.001$) and a significant interaction between temperature and treatment ($F(12,440)=7.55$, $p<0.001$).

As demonstrated in Table 3.1.10, the mean number of nymphs produced by the control treatment was significantly different to the Rb *N. ribisnigri* biotype on cv. Saladin at all temperatures except 26.4°C. When comparing the control to the Rb biotype on cvs. Eluarde and Rotary, the numbers of nymphs produced were significantly different at all temperatures except 5.5 and 26.4°C. At 26.4°C all treatments produced similar numbers of offspring. Rb + Saladin appeared to be the superior aphid biotype and lettuce cultivar combination as it produced more nymphs compared to the same biotype on Rotary at 5.5 and 12.5°C, and on Eluarde at 5.5, 12.5 and 15.9°C.

As presented in Table 3.1.11, the achieved fecundity recorded for the control did not differ significantly between temperatures. However, significantly more variation was observed between temperatures for each of the Rb biotype treatments. At 5.5 and 26.4°C, Rb biotypes demonstrated the lowest fecundity.

Table 3.1.10 Mean achieved fecundity provided by the ANOVA analysis for each treatment at 5.5, 12.5, 15.9, 21.4 and 26.4°C; n= the number of observations; *Tn*= total number of possible observations. Treatment means with different letters in a **row** are significantly different at that temperature.

	Treatments								
°C	n	**WT + Saladin (control)**	n	**Rb + Saladin**	n	**Rb + Eluarde**	n	**Rb + Rotary**	*Tn*
5.5	13	17.48[a]	17	32.71[b]	14	11.56[a]	14	12.15[a]	20*
12.5	25	25.08[a]	26	66.09[c]	27	47.52[b]	28	43.70[b]	30^
15.9	25	15.79[a]	30	38.57[b]	30	50.87[c]	29	42.41[bc]	30^
21.4	21	18.43[a]	24	43.24[b]	27	41.67[b]	26	34.76[b]	30^
26.4	12	15.70[a]	29	21.89[a]	27	14.76[a]	25	20.96[a]	30^

* LSD used for comparisons between different treatments at the same temperature which have minimum number of observations e.g. 5.5 (*Tn*20-20) = 0.04070

^ LSD used for comparisons between different treatments at the same temperature which have minimum number of observations e.g. 12.5 (*Tn*30-30) = 0.03640

Table 3.1.11 Mean achieved fecundity provided by the ANOVA analysis for each treatment at 5.5, 12.5, 15.9, 21.4 and 26.4°C; n= the number of observations; *Tn*= total number of possible observations. Treatment means with different letters in a **column** are significantly different between temperatures.

	Treatments								
°C	n	WT + Saladin (control)	n	Rb + Saladin	n	Rb + Eluarde	n	Rb + Rotary	*Tn*
5.5	13	17.48^{a}	17	32.71^{acde}	14	11.56^{a}	14	12.15^{a}	20*
12.5	25	25.08^{a}	26	66.09^{b}	27	47.52^{be}	28	43.70^{be}	30^
15.9	25	15.79^{a}	30	38.57^{a}	30	50.87^{ce}	29	42.41^{ce}	30^
21.4	21	18.43^{a}	24	43.24^{a}	27	41.67^{de}	26	34.76^{de}	30^
26.4	12	15.70^{a}	29	21.89^{cde}	27	14.76^{a}	25	20.96^{a}	30^

* LSD used for comparisons within a treatment between temperatures with minimum and maximum observations e.g. 5.5 and 12.5° (*Tn*20-30)= 10.620

^ LSD used for comparisons within a treatment between temperatures with maximum observations i.e. 12.5 and 15.9 (*Tn*30-30) = 8.671

When re-analysing the data after the alate data has been converted to missing values, the ANOVA produced a similar output, showing that, overall, there was a significant effect of temperature on fecundity ($F(4,9)=12.69$, $p<0.001$). There was also a significant effect of treatment ($F(3,402)= 47.31$, $p<0.001$), and a significant interaction between temperature and treatment ($F(12,402)=7.91$, $p=<0.001$). The increase in significance for the overall effect of temperature is due to an increase in the variation between the treatments and a reduction in the between-replicate variation.

Intrinsic rate of increase (r_m)

Figure 3.1.12 illustrates the mean r_m at each temperature for each of the six treatments. The mean r_m for the control treatment at 5.5, 12.5, 15.9, 21.4 and 26.4°C is 0.063, 0.156, 0.213, 0.304 and 0.279 respectively. For all treatments (excluding

WT + Eluarde and WT + Rotary) the r_m value increased with increasing temperature up to 26.4°C. At 26.4°C, there was more variation in the r_m value compared with the other temperatures and only one treatment (Rb + Rotary) showed an increase in the r_m value.

While several aphids survived to adulthood in the WT + Eluarde and Rotary treatments as shown in Table 3.1.7, only one WT biotype at 12.5°C on cv. Eluarde survived long enough for the r_m to be determined. This was calculated as 0.087 which is similar to the mean r_m recorded at 5.5°C rather than 12.5°C.

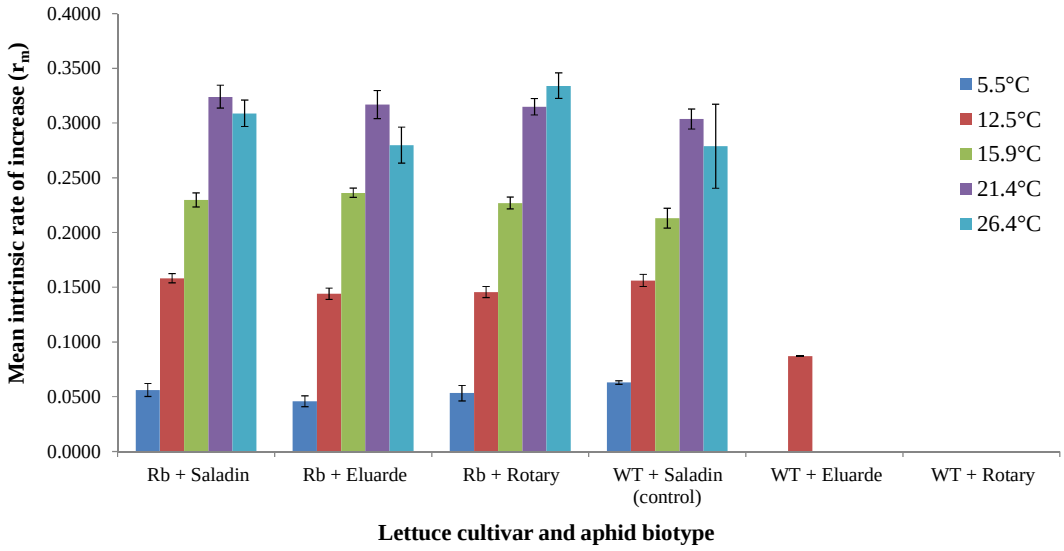


Figure 3.1.12 Mean intrinsic rate of increase (r_m) for each treatment at five constant temperatures (5.5, 12.5, 15.9, 21.4 and 26.4°C) with SE.

An ANOVA was performed on the pooled data for apterous and alate aphids (excluding WT + Eluarde and WT + Rotary treatments). This displayed a significant overall effect of temperature ($F(4,9)=80.76$, $p<0.001$) and treatment ($F(3,316)=2.77$, $p=0.042$) on the r_m. A significant interaction between temperature and treatment ($F(12,316)=2.89$, $p<0.001$) was also observed.

When using the LSD to compare r_m values between treatments at the same temperature, the control only differed from Rb + Saladin at 21.4°C, where the Rb biotype had a significantly higher r_m value (Table 3.1.13). At 5.5 and 12.5°C there were no differences between any of the treatments. As temperatures increased from 12.5°C, variation between the control and Rb biotype treatments occurred, with the

Rb biotypes having higher r_m values compared with the control except for the Rb + Eluarde treatment at 26.4°C. From 5.5 to 21.4°C the Rb biotype treatments did not significantly differ from each other, but more variation between treatments occurred at 26.4°C.

Table 3.1.13 Mean r_m provided by the ANOVA analysis for each treatment at 5.5, 12.5, 15.9, 21.4 and 26.4°C; n= the number of observations; *Tn*= total number of possible observations. Treatment means with different letters in a **row** are significantly different at that temperature.

	Treatments								
°C	n	**WT + Saladin (control)**	n	**Rb + Saladin**	n	**Rb + Eluarde**	n	**Rb + Rotary**	*Tn*
5.5	4	0.064a	12	0.056a	8	0.046a	4	0.056a	20*
12.5	15	0.155a	24	0.158a	22	0.142a	27	0.145a	30^
15.9	9	0.207a	20	0.227ab	27	0.234b	23	0.226ab	30^
21.4	10	0.298a	21	0.319b	22	0.320b	16	0.315ab	30^
26.4	10	0.304a	28	0.309acd	23	0.275b	20	0.327cd	30^

*LSD used for comparisons between treatments at the same temperature which has minimum observations e.g. 5.5 (*Tn*20-20) = 0.0256

^LSD used for comparisons between treatments at the same temperature with maximum observations e.g. 12.5 (*Tn*30-30) = 0.0209

When comparing the effect of temperature within each treatment (Table 3.1.14), at 5.5, 12.5 and 15.9°C, each temperature had a significantly different r_m value to the neighbouring temperature. However, at 21.4°C, for the control, Rb + Saladin and Rb + Rotary treatments the r_m was similar to those at 26.4°C, and for Rb + Saladin and *Rb* + Eluarde the r_m was lower at 26.4 than 21.4°C.

It should also be noted that in addition to the control exhibiting poor survival to adulthood as displayed in Table 3.1.7, aphids continued to die before their r_m could

be calculated, reducing the number of observations further, suggesting the control also had poor survival during the reproductive period.

Table 3.1.14 Mean r_m provided by the ANOVA analysis for each treatment at 5.5, 12.5, 15.9, 21.4 and 26.4°C; n= the number of observations; *Tn*= total number of possible observations. Treatment means with different letters in a **column** are significantly different between temperatures.

	Treatments								
°C	n	**WT + Saladin (control)**	n	**Rb + Saladin**	n	**Rb + Eluarde**	n	**Rb + Rotary**	*Tn*
5.5	4	0.064a	12	0.056a	8	0.046a	4	0.056a	20*
12.5	15	0.155b	24	0.158b	22	0.142b	27	0.145b	30^
15.9	9	0.207c	20	0.227c	27	0.234c	23	0.226c	30^
21.4	10	0.298de	21	0.319de	22	0.320d	16	0.315de	30^
26.4	10	0.304e	28	0.309e	23	0.275e	20	0.327e	30^

*LSD used for comparisons within a treatment between temperatures with minimum and maximum number of observations e.g. 5.5 and 12.5°C (*Tn*20-30) = 0.0425

^LSD used for comparisons within a treatment between temperatures with maximum number of observations i.e. 12.5 and 15.9 (*Tn*30-30) = 0.0380

When re-analysing the data converting the alate counts to missing values, the ANOVA produced a similar output, showing that overall, temperature ($F(4,9)=79.55$, $p<0.001$), and treatment ($F(3,289)=3.58$, p0.014), had a significant effect on r_m. There was also a significant interaction between temperature and treatment ($F(12,289)=3.44$, $p<0.001$). Excluding the alate data resulted in a more significant effect of treatment on the r_m, due to an increase in the variation between the treatments and a reduction in the between-replicate variation.

Aphid morph

The numbers of alate forms produced by each treatment were recorded (Table 3.1.15). Although the number of aphids (n) that the data were based on was low for some treatments, a general linear model (GLM) was used to model binomial proportions (e.g. logistic regression) which suggested that there was a significant effect of temperature ($F(4,7)=7.48$, $p=0.011$), but not treatment, on the number of alates produced.

Table 3.1.15 Number of alates produced by each treatment and the number of observations (n).

	Treatments							
	WT+Saladin		**Rb+Saladin**		**Rb+Eluarde**		**Rb+Rotary**	
°C	n	No. of alates	n	No. of alates	n	No. of alates	n	No. of alates
5.5	13	0	17	2	14	1	14	3
12.5	25	3	26	5	27	5	28	5
15.9	25	0	30	2	30	2	30	0
21.4	21	2	24	1	27	2	26	0
26.4	12	0	29	1	27	2	25	2

Figure 3.1.16 illustrates the significant effect of temperature, using the predicted means calculated from the logistic regression. A temperature of 12.5°C resulted in the highest number of alates. Using 5% LSDs to make comparisons between the means indicated that significantly more alates were produced at 12.5°C than at 15.9, 21.4 or 26.4°C.

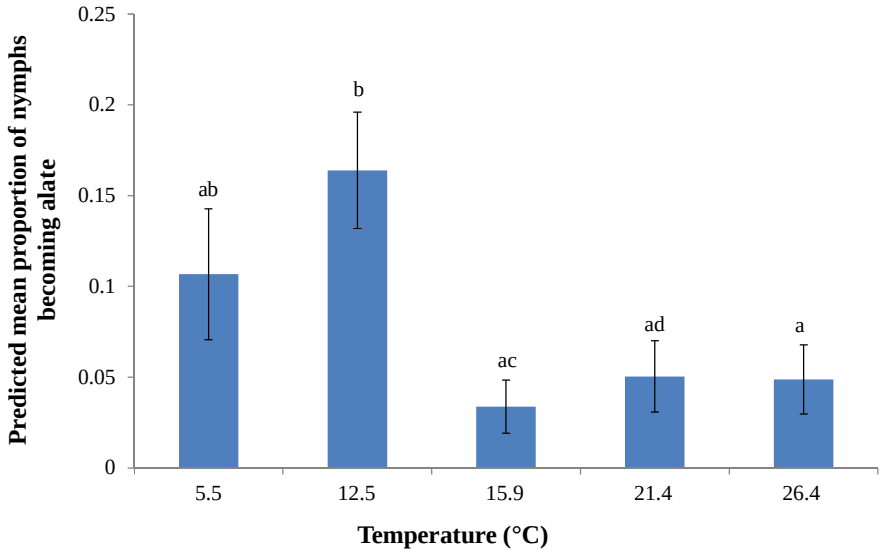


Figure 3.1.16 Predicted mean proportion of nymphs becoming alate for each temperature (5.5, 12.5, 15.9, 21.4 and 26.4°C) with SE. Treatment means with the same letter are not significantly different.

Figure 3.1.17 demonstrates that the WT and Rb *N. ribisnigri* biotypes performed similarly in terms of the proportion of alates produced.

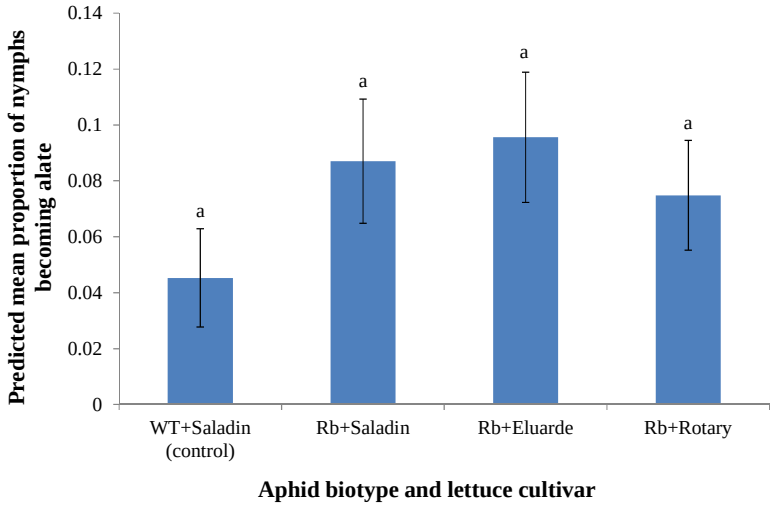


Figure 3.1.17 Predicted mean proportion of nymphs becoming alate for each of the four treatments with SE. Treatment rearing regimes with the same letter are not significantly different.

3.2 Effects of temperature on WT *N. ribisnigri*

Temperature recordings were made during each development experiment using Tiny Tags© to determine the deviation from the temperatures at which the rooms were set (10, 15, 20 and 25°C). The mean temperature recorded during each experiment was used as the treatment temperature to improve estimations from the development data. The revised treatment temperatures were 12.3, 15.7, 20.3 and 25°C.

Development time, r_m and number of alates

Data collected from Experiment 3.2 for the WT *N. ribisnigri* biotype on cv. Saladin are summarised in Table 3.2.1. Mean development times and the r_m were similar to those recorded in Experiment 3.1. The largest variation was observed for the r_m value at 25°C, which is likely to be because only 12 aphids provided the data in Experiment 3.1 compared with the 58 aphids in Experiment 3.2.

The number of alates occurring at each temperature was also recorded. The highest number of alates was recorded at 20.3°C, followed by 15.7°C. A two-sample binomial test showed a significant difference only between 25°C (proportion alate 0.017) and 20.3°C (proportion alate 0.121, p=0.028), and 25°C and 15.7°C (proportion alate 0.107, p=0.046).

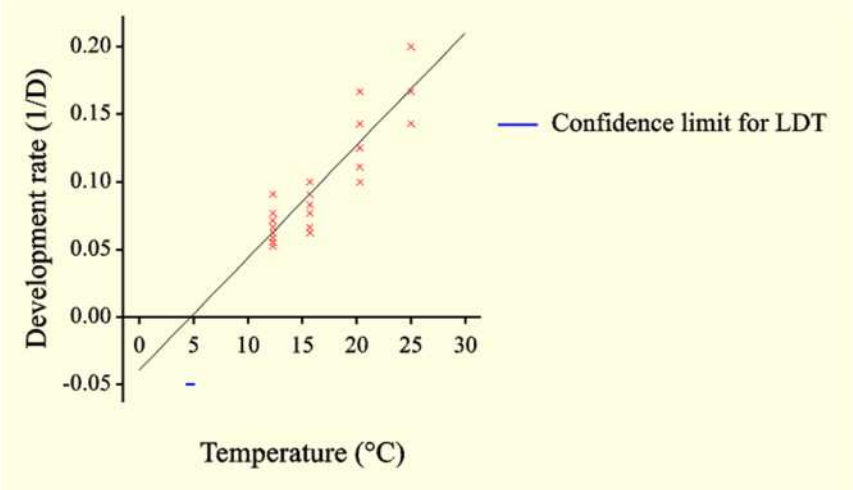
Table 3.2.1 Mean development time (±SE), mean r_m (±SE) and the number of WT+Saladin alates at 12.3, 15.7, 20.3 and 25°C. n= number of observations.

Temperature (°C)	Mean development time (days)	n	Mean r_m	n	Num. of alates
12.3	16.14 ±0.201	59	0.153 ±0.002	58	3
15.7	11.21±0.146	56	0.192 ±0.009	37	6
20.3	7.57 ±0.089	58	0.301 ±0.005	51	7
25.0	6.02 ±0.039	58	0.332 ±0.005	58	1

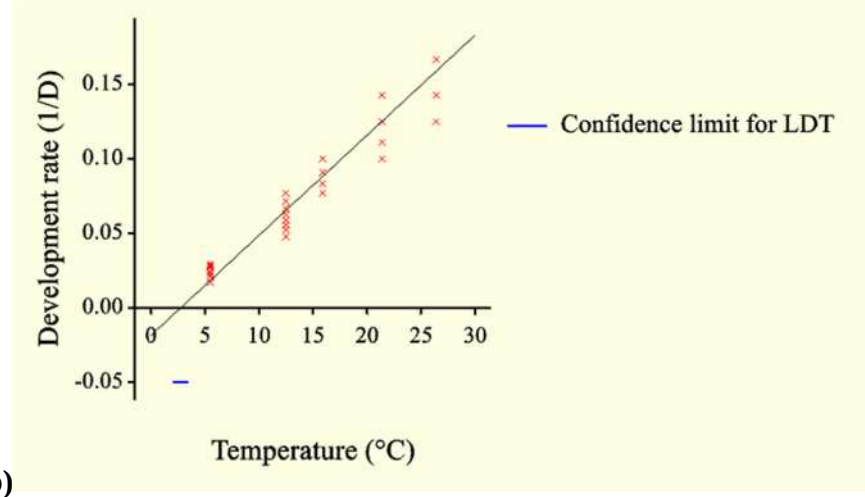
Developmental thresholds and day-degrees

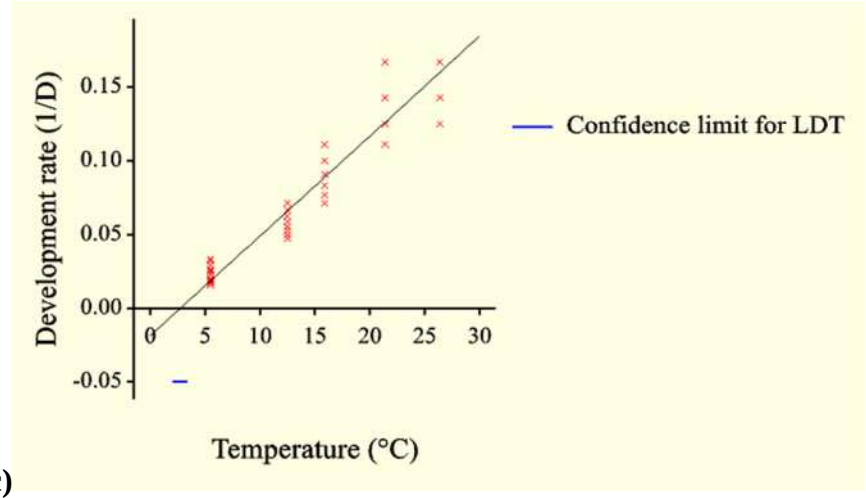
The linear regression of development rates (1/development time) to adulthood in relation to temperature for the above data was determined (Figure 3.2.2a). Linear regressions were also performed on the data for the WT and Rb *N. ribisnigri* biotypes on cv. Saladin at 5.5, 12.5, 15.9, 21.4 and 26.4°C, which were extracted from Experiment 3.1 (Figure 3.2.2b-c). These three data sets provided three estimates of the LDT and day-degree estimates for WT and Rb *N. ribisnigri* (Table 3.2.3).

a)



b)





c)

Figure 3.2.2 a-c Development rates (1/development time) to adulthood at different temperatures with the estimated LDT and 95% confidence limits **a)** WT + Saladin development rate data at 12.3, 15.7, 20.3 and 25°C from experiment 3.2 **b)** WT + Saladin development rate data at 5.5, 12.5, 15.9, 21.4 and 26.4°C from Experiment 3.1 **c)** Rb + Saladin development rate data at 5.5, 12.5, 15.9, 21.4 and 26.4°C from Experiment 3.1.

Table 3.2.3 Linear regression model, LDT (±SE), upper and lower 95% confidence limits (CL) and day-degree (DD) estimates for the linear regressions displayed in Figure 3.2.2 a-c.

Treatment	LDT (°C)	Upper CL	Lower CL	Linear regression model	DD
WT+Saladin (12.3-25°C)	4.724 ±0.21	5.140	4.308	Y= -0.03927+0.008312x	120.31
WT+Saladin (5.5-26.4°C)	2.777 ±0.36	3.488	2.066	Y= -0.01867+0.006722x	148.77
Rb+Saladin (5.5-26.4°C)	2.751 ±0.36	3.464	2.039	Y= -0.01860+0.006760x	147.93

3.3 Effects of aphid biotype on the propensity to become alate

The numbers of alate and apterous adults were recorded for WT *N. ribisnigri* biotype on cv. Saladin, and the Rb *N. ribisnigri* biotype on cv. Saladin and cv. Rotary (Table 3.3.1). A two-sample binomial (two-tailed) test was used to determine whether or not the proportions of alates in one treatment were equal to another treatment.

Table 3.3.1 Number of alate and apterous adult morphs recorded for each treatment and the number of observations (n).

Treatment	Alate	Apterous	n
WT + Saladin	0	30	30
Rb + Saladin	7	24	31
Rb + Rotary	7	25	32

When comparing the proportion of alates produced by the WT *N. ribisnigri* biotype on cv. Saladin (proportion 0.00) to those of the Rb *N. ribisnigri* biotype on cv. Saladin (proportion 0.23, p=0.006) and cv. Rotary (proportion 0.22, p=0.007) a significant difference in the proportions was observed. This suggests that the Rb biotype has a higher propensity to produce alate forms compared with the WT biotype. When the proportions of alates produced by the Rb *N. ribisnigri* biotypes on cv. Saladin and Rotary were compared, no differences were identified, suggesting that the type of cultivar has no influence on the propensity to become alate.

3.4 Effect of photoperiod on the development of different aphid biotypes

Development time

Figure 3.4.3 illustrates the mean development time to adult under each of the three rearing regimes (20°C 14L:10D, 20°C 16L:8D and 15°C 14L:10D) for each of the 10 treatments. As expected, development time to adult took the greatest number of days at 15°C compared with 20°C. None of the WT *N. ribisnigri* biotypes survived to adulthood on the resistant cv. Rotary. The control (WT+Saladin) had a mean

development time of 7.76 days at 20°C 14L:10D, 7.25 at 20°C 16L:8D and 11.73 at 15°C 14L:10D.

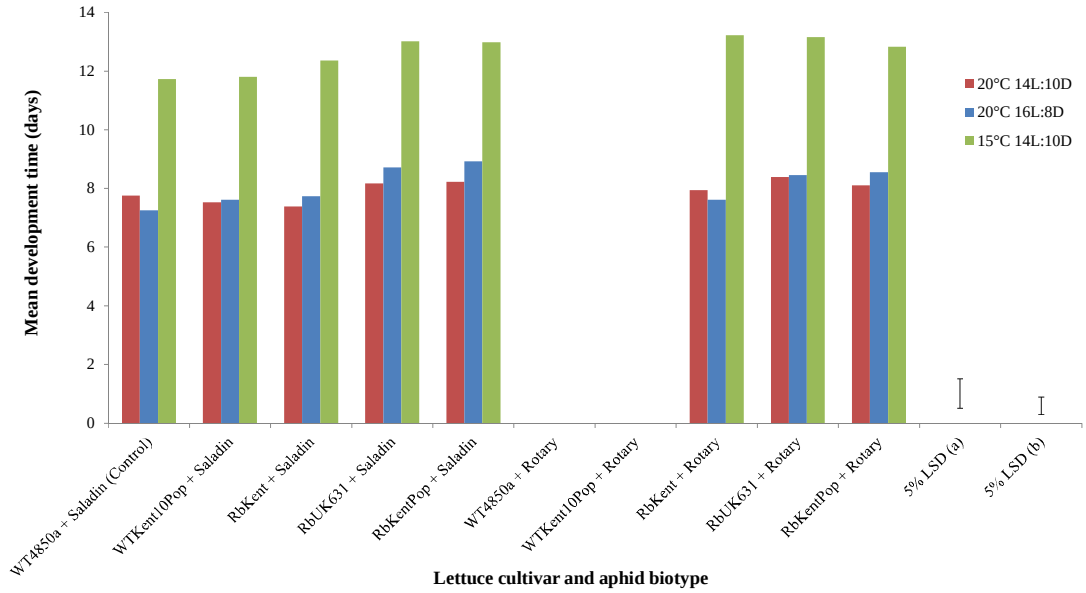


Figure 3.4.3 Mean development time to adult in days for the 10 aphid biotype and lettuce cultivar treatments under three rearing regimes (20°C 14L:10D, 20°C 16L:8D and 15°C 14L:10D). 5% LSDs are shown for (a) comparisons between lettuce cultivar and aphid biotype treatments at different rearing regimes (b) comparison between lettuce cultivar and aphid biotype treatments within the same rearing regime.

An ANOVA was performed on the pooled data for apterous and alate aphid morphs. This indicated a significant overall effect of rearing regime on the development time ($F(2,6)=99.60$, $p<0.001$). There was also a significant effect of aphid biotype ($F(4,317)=31.81$, $p<0.001$). Interactions were observed between aphid biotype and rearing regime ($F(8,317)=4.22$, $p<0.001$), lettuce cultivar and rearing regime ($F(2,317)=4.63$, $p=0.01$) and aphid biotype and lettuce cultivar ($F(2,317)=3.48$, $p=0.032$). Lettuce cultivar did not have an effect on development time.

When using the LSDs to compare development times between the same treatments at 20°C 14L:10D and 20°C 16L:8D, there was no significant difference, suggesting that photoperiod does not have an effect on development time. Variation in development

time was observed when comparing the 10 treatments under the same rearing regime.

At 20°C 14L:10D, the control was significantly different to the RbUK631 biotype on cv. Rotary. At 16L:8D 20°C, the control was significantly different to aphid biotypes RbUK631 and RbKentPop on both cultivars. At 15°C 14L:10D, the control was significantly different to all the Rb biotypes on both cultivars. The two WT *N. ribisnigri* biotypes performed similarly at each rearing regime.

When comparing the development times of the RbKent biotype on cv. Saladin against the other Rb aphid biotypes, variation was also observed. At 20°C 16L:8D and 20°C 14L:10D, RbKent on cv. Saladin was significantly different to RbUK631 and RbKentPop on both cultivars. At 15°C 14L:10D, RbKent on cv. Saladin was significantly different to RbUK631 on both cultivars, RbKentPop on Saladin and RbKent on Rotary. This indicates that there was variation between the Rb *N. ribisnigri* biotypes under each rearing regime.

Intrinsic rate of increase (r_m)

Figure 3.4.4 illustrates the mean r_m under each light and temperature rearing regime for each of the 10 treatments. The control (WT+Saladin) had a mean r_m of 0.316 at 20°C 14L:10D, 0.334 at 20°C 16L:8D and 0.183 at 15°C 14L:10D.

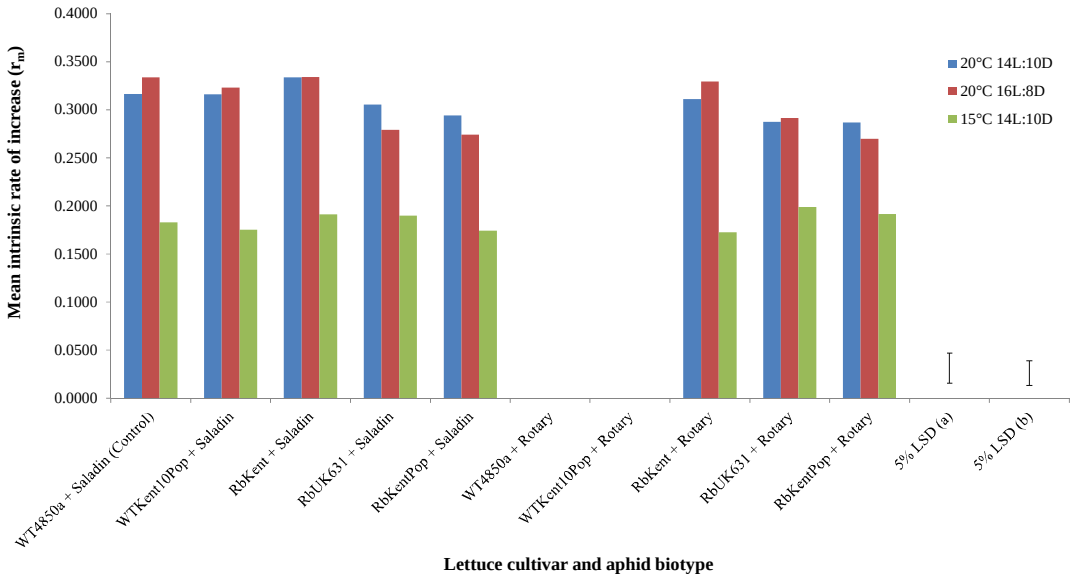


Figure 3.4.4 Mean r_m for the 10 aphid biotype and lettuce cultivar treatments under three rearing regimes (20°C 14L:10D, 20°C 16L:8D and 15°C 14L:10D). 5% LSDs are shown for (a) comparisons between lettuce cultivar and aphid biotype treatments under different rearing regimes (b) comparison between lettuce cultivar and aphid biotype treatments within the same rearing regime.

An ANOVA was performed on the pooled data for apterous and alate aphid morphs. This indicated a significant overall effect of rearing regime on the r_m ($F(2,6)=122.05$, $p<0.001$). There was also a significant effect of aphid biotype ($F(4,245)=10.70$, $p<0.001$), and the interaction between aphid biotype and rearing regime ($F(8,245)=6.33$, $p<0.001$). Lettuce cultivar did not have an effect on r_m.

When using the LSDs to compare the r_m between the same treatments at 20°C 14L:10D: and 20°C 16L:8D there was no significant difference. Variation in r_m was observed when comparing the 10 treatments under the same rearing regimes, indicating that the different aphid biotypes respond differently under each rearing regime. At 20°C 14L:10D, the control was significantly different to the RbUK631 and RbKentPop biotypes on cv. Rotary. At 20°C 16L:8D, the control was significantly different to the aphid biotypes RbUK631 and RbKentPop on both cultivars. At 15°C 14L:10D, the control was not significantly different from any of the other treatments and overall much less variation was seen between treatments

under this rearing regime. Under each rearing regime, the control performed similarly to the other WT *N. ribisnigri* biotype (WTKent10) and also to the RbKent biotype on both cultivars. The RbUK631 and RbKentPop aphid biotypes were responsible for most of the variation between treatments under each rearing regime in 20°C 14L:10D and 20°C 16L:8D, which when compared with the control had lower r_m values.

When comparing the r_m of RbKent on Saladin against the other Rb aphid biotypes, variation was also observed. At 20°C 16L:8D and 20°C 14L:10D, the biotype was significantly different to RbUK631 and RbKentPop on both cultivars. At 15°C 14L:10D, no differences were observed. This indicates that there was variation in the r_m between the Rb *N. ribisnigri* biotypes, but only at 20°C.

Aphid morph

The numbers of alate forms produced under each rearing regime for each treatment were recorded (Table 3.4.5).

Table 3.4.5 Number of alates produced at each treatment and the number of observations (n).

	Rearing regime					
	20°C 14L:10D		**20°C 16L:8D**		**15°C 14L:10D**	
Treatment	**n**	**Num. of alate**	**n**	**Num. of alate**	**n**	**Num. of alate**
WT4850a + Saladin	16	0	16	0	14	0
WTKent10pop + Saladin	16	4	18	0	15	6
RbKent + Saladin	18	0	16	1	17	1
RbUK631 + Saladin	17	1	16	3	12	1
RbKentPop + Saladin	17	1	17	0	15	0
RbKent + Rotary	16	2	18	2	16	3
RbUK631 + Rotary	18	3	17	5	16	1
RbKentPop + Rotary	16	3	18	0	16	1

Although the total number of aphids (n) was low for some treatments, a general linear model was used to model binomial proportions (e.g. logistic regression). No effect of rearing regime on the proportion becoming alate was observed ($F(2,4)=1.21$, $p=0.388$). However, a significant effect of treatment (aphid biotype and lettuce cultivar) was observed ($F(7,42)=3.46$, $p=0.005$) as illustrated in Figure 3.4.6.

The control treatment (WT + 4850a) did not produce any alates, while the WTKent10pop biotype produced the most alates. Alate aphids occurred in all the Rb aphid treatments, with RbUK631 producing the most alates on both lettuce cultivars with more alates occurring on cv. Rotary. The most variation was observed between the Rb biotypes on cv. Saladin.

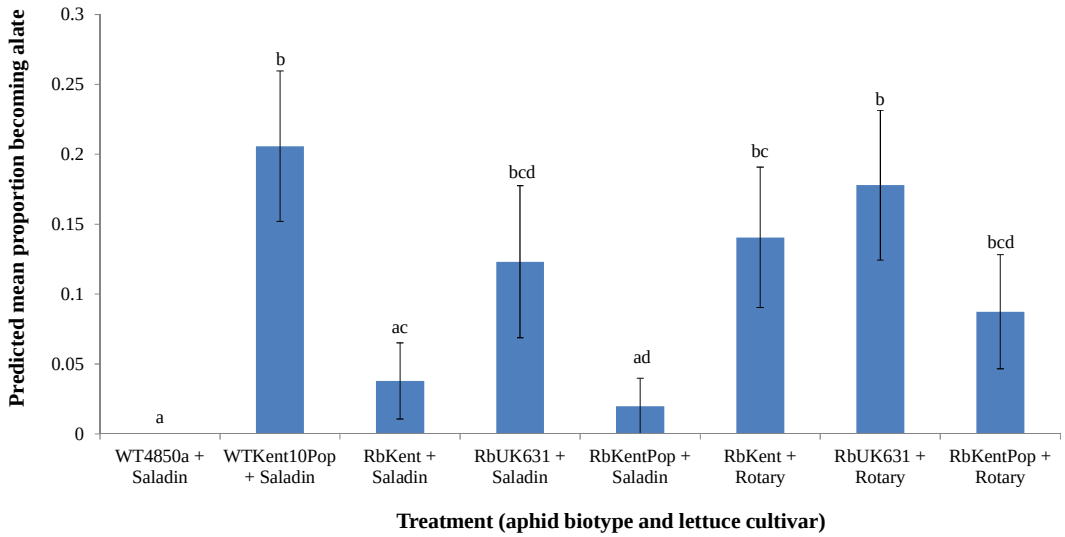


Figure 3.4.6 Predicted mean proportion of nymphs becoming alate under each rearing regime with SE. Rearing regimes with different letters are significantly different.

Discussion

The effects of temperature on the developmental parameters of *N. ribisnigri* were first investigated by Diaz and Fereres (2005), using nymphs collected in Spain. Diaz and Fereres (2005) recorded mean development times of 31.5, 15.8, 11.5, 8, 6.5, 6.3, and 7 days at 8, 12, 16, 20, 24, 26 and 28°C (14L:10D) respectively. They also

determined that the optimum temperature for population increase was between 20 and 24°C through calculation of the r_m.

In this study, the optimum temperature for population increase identified for UK WT *N. ribisnigri* was 21.4°C, which is within the range identified by Diaz and Fereres (2005). The mean development times recorded by Diaz and Fereres at 12, 16, 20 and 26°C also closely reflected those recorded in this study at 12.5, 15.9, 21.4 and 26.4°C at 16L: 8D, suggesting that the different photoperiods used in the two studies did not influence development time. The lack of effect of photoperiod was also observed within this study, where no effects of different photoperiods (14L: 10D and 16L: 8D) were observed on the development time or $r_{m.}$

Characteristically, and as described for many aphid species, in this study the development time of *N. ribisnigri* decreased with increasing temperature. However, at 26.4°C the size of this decrease was much smaller than the successive differences between the lower temperatures, suggesting that 26.4°C is approaching the UDT. This was also observed for the rate of population increase where at temperatures lower than 26.4°C the r_m had been increasing with temperature, but decreased at 26.4°C. This was also observed by Diaz and Fereres (2005), where their estimates of r_m at 8, 12, 16, 20, 24 and 26°C (14L:10D) were 0.074, 0.127, 0.224,0.332, 0.372 and 0.173 respectively, with a reduction in the r_m at 26°C. It is likely that the inclusion of an even higher temperature treatment in the present study would, compared with 26°C, have resulted in an increase in development time, increased mortality and halted/reduced reproductive output as observed by Diaz and Fereres (2005) with a treatment at 28°C. Deleterious effects of high temperatures such as these have been observed for other aphid species (Kenten, 1955; Dixon, 1989; Acreman and; Barlow, 1962;).

At 5.5°C, which was the lowest temperature used in this study, aphids demonstrated the longest development times and lowest r_m values, which varied widely between individuals. Furthermore, at this temperature aphids exposed to all treatments had low survival (between 43.3% and 56.7%), indicating that this temperature is nearing the LDT. The variability between individuals at this temperature could be due to the natural variation in the ability of individuals within a population to survive at lower temperatures (Griffiths and Wratten, 1979).

Although the r_m values estimated in this study were similar to those determined by Diaz and Fereres (2005), it is uncertain whether the estimates are comparable, as, in the present study, the D value representing the pre-reproductive period was calculated from birth to the final adult moult (Awmack and Leather, 2007), while other studies, including Diaz and Fereres (2005), use the time from birth to the onset of reproduction (Wyatt and White, 1977). The latter approach takes into account that there can be delay in the birth of the first nymph following the final adult moult in aphids (Dixon and Wratten, 1971).

The data in this study indicated that the onset of reproduction was delayed at 5, 10, 15 and 20°C, while at 25°C newly-moulted adults were usually found with nymphs. Reproduction was delayed for approximately one day at 15 and 20°C and two days at 10°C. At 5°C the delay was variable and as the aphids were not monitored after they had reached adulthood, an estimate of the reproductive delay could not be determined. Recalculating the r_m by extending the D time to include the estimated reproductive delays did not provide better estimates when compared to the Diaz and Fereres (2005) study, which is likely to be due to undercounting the offspring as data were not available to determine the number of nymphs produced for the period equivalent to the estimated D. As the reproductive delays were only small at 10, 15 and 20°C it is thought that the original r_m estimates are likely to be only marginally different from what they would have been if the alternative method had been used.

In this study the control treatment (WT biotype on cv. Saladin) demonstrated consistently poor survival at all the temperatures considered when compared to the Rb biotype treatments. Therefore it is proposed that cv. Saladin could possess partial resistance to the WT *N. ribisnigri* biotype.

All plants have adapted various defensive strategies to respond to insect attack and demonstrate a range of antibiotic and antixenotic mechanisms (see Chapter 7) which can include cell wall modifications, defensive proteins and secondary metabolites (Goggin, 2007). As a result, host plants have a range of susceptibilities and cv. Saladin may be a less suitable host plant for the WT biotype compared with the other cultivars screened. Possible reasons for this could be due to cv. Saladin having a low nitrogen content which has been observed in other studies to result in poor development, fecundity and survival of aphids (Prosser and Douglas, 1992; Awmack

and Leather, 2002). Furthermore, response to low nitrogen can vary between clones. For example, some clones of *A. fabae* had depressed performance when fed on diets lacking certain amino acids while others were unaffected (Wilkinson and Douglas, 2003).

Previous host plant experience can also influence the performance of an aphid on another host. For example, five *A. pisum* clones were found to display varying levels of performance when transferred to a different host plant to the one they were collected from, suggesting they were better physiologically/behaviourally adapted to their original host plant (Sandström and Pettersson, 1994). In the present study, *N. ribisnigri* did not have a choice of host plant. In future work, it would be interesting to perform a preference test to determine whether cv. Saladin is a less preferred host plant compared with a more susceptible line.

One of the most interesting observations from this study was the survival of WT biotypes on Nr-gene lettuce cultivars at 5.5, 12.5, and 15.9°C where 100% mortality would have been expected. While the aphids survived, their fecundity, development times and survival were adversely affected, indicating that cross-contamination of the cultures had not occurred, as an Rb biotype would have displayed development parameters comparable to the control. Therefore, at lower temperatures, it appears that the Nr-gene in the resistant cultivars fails to provide complete resistance against *N. ribisnigri*. This suggests that the Nr-gene could be temperature sensitive, and similar observations have been made for genes involved in plant-virus resistance. For example, L genes (L^1, L^2, L^3 and L^4) providing resistance to *Tobamovirus* pathotypes P_0 in peppers are temperature-sensitive and fail to provide resistance at higher temperatures (e.g. 30°C) (Matsumoto, *et al.*, 2008). More recently, Matsumoto *et al.* (2008) identified a new L gene (L^{1a}) which when expressed homozygously provides resistance to P_1 classified *Tobamoviruses* at 24°C but not 26 and 28°C. This reference to plant-virus interactions is relevant as it has been suggested that the gene-for-gene interaction described for plants and pathogens, could also be applied to the aphid-plant interactions where plant resistance genes recognise the avirulence gene in the insect (Kaloshian, 2004).

The Rb biotype demonstrated similar development times and r_m values, irrespective of host plant when compared to the control, indicating that it is unaffected by the

resistance mechanism controlled by the Nr-gene. However, as already discussed cv. Saladin may not have been a suitable control host plant if it was providing partial resistance to the WT biotype, and had a more susceptible cultivar been used, differences in performance may have been observed between the WT and Rb biotypes. Other studies on non-UK Rb biotypes have shown depressed performance compared to the WT biotype when the aphids are reared on susceptible cultivars, suggesting that there is a trade-off between being able to overcome the resistance provided by the Nr-gene and development on susceptible lettuce cultivars (Smilde, *et al.*, 2009).

Trade-offs have been discussed extensively in the literature with regard to the cost of insecticide resistance. For example, studies have shown that insecticide-resistant clones of *M. persicae* may have increased susceptibility to parasitism due to a reduced response to aphid alarm pheromone and a reduced ability to overwinter (Foster, *et al.*, 1996; 1997; 2007; 2011). Therefore it is possible that the Rb biotypes which overcame Nr-gene host plant resistance could have their fitness reduced in other respects. Such fitness trade-offs could be beneficial in constraining the spread of the Rb biotype, particularly if it had reduced fitness in the winter and it was confirmed that this biotype overwintered as live aphid where it would be susceptible to low temperatures. Despite this, little research has addressed the trade-offs when aphids overcome host plant resistance.

In both Experiments 3.1 and 3.3, the Rb biotype produced more alate aphids compared to the WT biotype, which could have important implications for the dispersal potential of this new biotype. Many factors may influence the induction of alates including crowding, host plant nutrition, interaction with natural enemies and temperature (Müller, *et al.*, 2001).

In these experiments, nymphs were obtained by releasing a number of alates into a cage of lettuce plants for 24 hours to produce offspring, which were then reared individually to adulthood on lettuce plants. Therefore, variation in the propensity of the WT and Rb biotypes to produce alates could be the result of differences in pre-natal crowding, as the crowding history of the parent is unknown.

Furthermore, as the Rb biotype in Experiment 3.1 demonstrated higher fecundity compared to the WT biotype, variation in early post-natal crowding (<24 hours)

could have occurred also, as the Rb offspring may have been surrounded by more sisters than the WT biotype. To clarify this, further experiments would need to be carried out to take account of the possible effect of variation in pre-natal and early post-natal crowding.

Investigations into the propensity to produce alates have indicated that there is both within and between clonal genetic variation in response to the same environmental cues (Braendle, *et al.*, 2005; Hazell, *et al.*, 2005), which in the pea aphid has been related to aphid colour with red clones producing more alates compared to green clones (Weisser and Braendle, 2001). Sutherland (1969) also observed that the type of host plant could influence the proportion of alates produced by the pea aphid. During Experiment 3.4, the numbers of alates produced by the four Rb *N. ribisnigri* biotypes varied and were also influenced by the host plant, with cv. Rotary resulting in more alates compared to those on cv. Saladin.

In this study, temperature had the most significant influence on the production of alates by both WT and Rb biotypes, but the results were inconsistent between experiments. During Experiment 3.1, the most alates were produced by WT and Rb *N. ribisnigri* at 12.5°C, while in Experiment 3.2 the most alates were produced by WT *N. ribisnigri* at 20.3°C. While this could suggest that other factors apart from temperature were influencing alate induction and varied between the studies, it is also possible that the small sample size in Experiment 3.1 resulted in the inconsistency. Therefore more replicates would be required to confirm this observation. Similar work on *N. ribisnigri* also observed a strong effect of temperature on the proportion of alate aphids produced, but found that temperatures lower than 16°C led to the production of mainly apterous aphids (Diaz and Fereres, 2005). While one might expect that higher temperatures would result in more alates as a result of increased activity causing more tactile stimulation, many studies including this one, have shown that high temperatures are more likely to result in apterous morphs, with lower temperatures favouring alate induction (Müller, *et al.*, 2001). It has been proposed that this strategy acts to maximise dispersal efficiency during periods when other alate induction factors such as crowding or poor host plant quality are not predominant (Liu, 1994).

Analysing the developmental parameters of alate and apterous morphs separately or together in Experiment 3.1 did not affect the outcome of the statistical analysis, which can be attributed to the low number of alates analysed. If data based on larger numbers of alates were available, a significant difference might have been observed, as it is well documented in the literature that the development of wings results in negative effects on development and reproduction due to some of the energy being diverted for the development of wings (Dixon, *et al.*, 1993a; Zhang, *et al.*, 2009).

When estimating the LDT and DD from birth to adulthood for the WT biotype, it was concluded that Experiment 3.2 would have provided the most reliable estimates, as the data set provided by Experiment 3.1 was too small to represent variation between individuals. It was estimated that WT *N. ribisnigri* (apterous and alates combined) had a LDT of 4.7°C (lower confidence limit of 4.31°C and upper confidence limit of 5.14°C) and that development from birth to adulthood required 120.31 DD. Using the same method, Diaz *et al.* (2007) estimated an LDT of 3.6°C for apterae, 4.1°C for alate*s* and 3.14 combined, with DD of 125, 143 and 143 respectively. Therefore there is over a 1°C difference in the estimates between the two studies.

Differences between the studies could be the result of different aphid genotypes (inter-clonal variation) and host plant rearing procedures (Campbell and Mackauer, 1977), which as discussed in the Introduction can influence development thresholds, particularly when clones are collected from different geographic origins and are adapted to different climates. While this may explain some of the differences when comparing the two studies, it is also possible that that an overestimation of the LDT and DD occurred in the current study due to the extrapolation of the LDT being based on data from only four temperatures. In contrast, Diaz et al (2007) used data from every 3°C change, which would have provided a more accurate linear estimation of the LDT. Furthermore, the linear extrapolation in this study was derived from mainly moderate to high temperatures, and while this is common when using this extrapolation method, as determining development rates at lower temperatures is confounded by time (Liu and Meng, 1999), it has been exacerbated in these estimates as development rates between 5 and 10°C were not investigated. To refine the LDT and DD estimates further, development rates should be determined for additional temperatures between 5-20°C. Future work, could also

determine whether the estimates between Rb and WT *N. ribisnigri* biotypes differ and whether they are influenced by the geographic origin of the clones.

When investigating the effects of photoperiod (14L:10D and 16L:8D) on the four Rb biotypes and two WT biotypes, no differences were observed at 20°C. However, within each rearing regime variation between the clones was observed. Most of this variation resulted from the RbUK631 and RbKentPop biotypes, which had significantly different development times and r_m values to the WT4850a and/or RbKent biotype. This suggests that inter-clonal variation can exist between biotypes.

A similar observation was also been made by Kilian and Nielson (1971) who observed variation in the development times and r_m values between populations of *A. pisum* collected from different geographical areas. Similarly, variation in survivorship and mean relative growth rates were observed in *Aphis craccivora* and *Acyrthosiphon kondoi* clones (populations raised from a single female) collected from a range of host plants and geographic areas when reared on several suitable host plants (Edwards, 2001). Therefore these studies also support variation exists and aphids from different environments often perform differently.

In conclusion, this study has provided information on the basic developmental parameters of the UK WT4850a and RbKent *N. ribisnigri* biotypes in response to temperature and photoperiod, with consideration of other influencing factors such as the host plant and photoperiod. It has provided LDT and DD estimates for the WT4850a biotype which could be used to refine current forecasts or refine the direction of future work. More importantly, this study has highlighted the need to analyse a range of clonal lines in future studies to capture the inter-/intra-clonal variation present within an aphid species to provide more representative population data.

Conclusion

- This study confirms that temperature is a significant factor affecting the developmental time, developmental rate, intrinsic rate of increase, fecundity and the propensity to become alate of both WT and Rb *N. ribisnigri*.
- Between 5.5 and 26.4°C, development time decreased with increasing temperature and only at 5.5°C did variation in development times occur with the control (WT4850a on cv. Saladin) being significantly different to Rb cv. Saladin and Rb cv. Rotary.
- At lower temperatures, some aphids from the WT4850a biotype survived on Nr-gene cultivars, although their longevity, fecundity and development time were compromised.
- The r_m increased with increasing temperature up to 26.4°C, where then r_m decreased or did not significantly increase further.
- The WT4850a and RbKent biotypes had similar development times and r_m values.
- The control treatment exhibited poor survival when compared to the Rb biotype, demonstrating poor pre-reproductive survival and continued poor survival during the reproductive phase, particularly at 5.5 and 26.4°C. The Rb biotype experienced comparable poor longevity only at 5.5°C. The optimum temperature for longevity was at 15°C.
- The control treatment exhibited consistent poor achieved fecundity. However, RbKent biotypes had significantly higher achieved fecundity than the control between 12.5 and 21.4°C. Achieved fecundity was reduced for the Rb biotype at 5.5 and 26.4 °C. Overall, the optimum temperature for reproduction was 12°C.
- Collectively, these responses suggest that 5.5 and 26.4°C are close to the UDT and LDT, where aphid performance is negatively affected, meaning that the temperature range leading to optimum aphid performance is between 12.5-21.4°C.
- More alates were produced at lower temperatures and the RbKent biotype appeared to produce more alates compared to the WT45850a biotype, but not the WTKent10Pop biotype.

- Photoperiod did not influence development time or the r_m value. However, inter-clonal variation was observed, particularly between the control treatment and RbUK631 and RbKentPop biotypes. This occurred at each rearing regime for development times, but only at 20°C 14L:10D and 20°C 16L:8D for the r_m value.
- Estimates of the LDT and DD suggest that the WT4850a *N. ribisnigri* biotype has a LDT of 4.7°C and requires 120.31 day-degrees to reach adulthood.

Chapter 4: Overwintering Biology

Introduction

Insects have evolved a range of effective strategies to survive the unpredictable and unfavourable conditions which occur during the winter months. These strategies are designed to promote survival through a period which threatens damage from low temperatures and potential starvation (Leather, *et al.*, 1993).

Depending on the species, insects overwinter in various developmental stages (egg, pupa, larva/nymph or adult) and for aphids, this can consist of more than one stage. Aphids which exhibit an anholocyclic life-cycle, and therefore lack a sexual phase, overwinter as live parthenogenetic nymphs and adults, developing and reproducing slowly throughout the winter, while holocyclic species, which undergo a sexual phase, produce overwintering eggs that diapause on the winter host plant (Williams and Dixon, 2007). The overwintering success of these stages is critical as this determines the size and phenology of insect populations occurring in the following spring and summer (Leather, *et al.*, 1993).

Producing overwintering eggs is considered the 'safest' strategy since they are more resistant to cold temperatures compared with active stages. For example, the eggs of a temperate specie such as *R. padi* have been reported to resist temperatures of -30ºC for up to one month (Strathdee, *et al.*, 1995). Furthermore, the sexual production of eggs provides an opportunity for genetic recombination to occur, providing new genotypes that will be subject to natural selection in the spring (Dixon, 1998). While eggs do suffer high natural mortality resulting from predation and unfavourable conditions leading to waterlogging and desiccation (Leather, 1981; Leather, *et al.*, 1993), overwintering adults and nymphs are subject to significant mortality during the winter as they are less cold-hardy. Following a mild winter, however, anholocyclic species can benefit from their ability to begin development and reproduction as soon as conditions are favourable in the spring (Powell and Bale, 2008). Therefore, it is not surprising that some aphid species, such as *M. persicae*, adopt both strategies in locations where winters can be mild (Vorburger, 2004).

Nasonovia ribisnigri has a holocyclic life-cycle and therefore overwinters as an egg, although there is evidence for the occurrence of anholocyclic clones (see Chapter 1). In response to deteriorating conditions in autumn, *N. ribisnigri* produces alate gynoparae and males which migrate to the winter host (*Ribes* species). The gynoparae then viviparously produce sexual egg laying females called oviparae, which mate with the males and lay eggs close to the dormant buds of the winter host.

Research into the cues which stimulate the production of the sexual forms of various aphid species, has confirmed that temperature, and particularly photoperiod, are the main factors which, when experienced by the mother (as an embryo up to two days before her own birth), result in the 'switch' (Lees, 1959; Lees, 1963; Lamb and Pointing, 1972). For some species additional factors contribute to the 'switch', for example, *P. bursarius* produces sexual morphs in response to the cessation of plant growth as well as decreases in temperature, but is not influenced by photoperiod (Williams and Dixon, 2007). By determining the requirements for production of sexual morphs and subsequent egg laying, researchers can carry out crosses between aphids and investigate their genetics (Komazaki, 1998). The information can also be used for the purposes of plant protection, through the development of forecasts of the timing of egg hatch in the spring (Graf, *et al.*, 2006).

Research into the effects of photoperiod has shown that it is the length of the scotophase which is the important stimulus for the production of sexual morphs, rather than the length of the photophase (Lees, 1973). As a result, various researchers have now determined the critical night lengths (CNL) required for various aphid species. These studies have demonstrated differences in the CNL between sexual morphs (Matsuka and Mittler, 1979; Harrington, 1984) and between aphid species and clones (Via, 1992; Vaz Nunes and Hardie, 2000b; Vaz Nunes and Hardie, 2000a). It has also been recognised that, for an aphid to be able to respond to changes in photoperiod, there must be a clock-counter system to sense night length and record information each day. While hour-glass and various circadian oscillatory based mechanisms have been proposed, currently none have been demonstrated (Lees, 1986; Hardie, 2009).

Through investigations of the effects of temperature on the production of sexual morphs, various influences on the photoperiodic responses have been observed.

When studying *Megoura viciae*, Lees (1959) observed that high temperatures suppressed male production, while low temperatures suppressed them partially. He observed that for non-feeding aphids, the CNL for production of oviparae was temperature-compensated (possessing a mechanism to compensate for changes in temperature so that the response to photoperiod remains 'accurate') between 6-20ºC (Lees, 1986), while starvation at 15°C for 4 hours at the beginning and end of the scotophase and photophase resulted in the CNL shortening, suggesting adequate nutrition could be required for the photoperiodic timer to function correctly (Lees, 1986). A difference in the accuracy of temperature-compensation mechanisms has also been observed between fasting and feeding aphids (Lees, 1963; Lees, 1986).

For *A. fabae* the CNL for production of gynoparae did not appear to be compensated well, changing by 1 h per 5ºC change in temperature, while for males, compensation was better changing only 0.5 h per 5ºC change, indicating that variation in temperature sensitivity exists between sexual morphs as well as species (Vaz Nunes and Hardie, 2000b). Therefore, it is not surprising that studies have also shown variation between clones in temperature sensitivity. For example, in Scottish clones of *M. viciae*, temperature compensation on the CNL for production of oviparae was less pronounced (increasing 2 h per 5ºC change) than in English clones (increasing 15 min per 5ºC change) (Vaz Nunes and Hardie, 2000a). This confirms that photoperiod, temperature, and possibly nutrition, play a role in production of sexual morphs, but the intra- and inter-specific responses of aphids to these factors indicate that the relationship is complex and often difficult to interpret. Therefore, no single condition can be used to induce sexual morphs under laboratory conditions for all aphid species.

Following the production of sexual morphs, mating occurs and eggs are produced. This is the stage during which the majority of temperate aphids enter diapause and, once again, studies have shown that photoperiod and temperature regulate this period (Tauber and Tauber, 1976).

Tauber *et al.* (1986) defines diapause as a 'neurohormonally mediated, dynamic state of low metabolic activity' which 'occurs during a genetically determined stage(s) of metamorphosis' and is 'usually in response to a number of environmental stimuli that precede unfavourable conditions'. It is widely accepted that diapause is an

alternative pathway to morphogenesis with its own metabolic demands (Hahn and Denlinger, 2011), and is made up of three phases known as pre-diapause, diapause and post-diapause (Koštál, 2006). The progression through each of these phases will be referred to as diapause development in this study.

Pre-diapause describes the induction phase where insects respond to certain diapause-inducing stimuli (Koštál, 2006), which for aphids, includes the response to changes in temperature and photoperiod to produce sexual morphs as previously discussed. Following this, the diapause phase begins which includes the sub phases of initiation, maintenance and termination (Koštál, 2006). For aphids the initiation phase is easily distinguished by the production of an overwintering egg which is maintained in diapause so that the state persists until certain physiological processes have occurred, even if favourable environmental conditions return. Therefore, once egg laying commences, the eggs are in diapause and are maintained in this state for an extended period, so that even if conditions favourable for egg development occur, the insect does not respond until winter has passed. Tauber *et al.* (1986) hypothesised that diapause is probably maintained via changes in thermal thresholds and/or remaining sensitive to the diapause-inducing stimuli, particularly photoperiod.

Eventually diapause is no longer maintained and is terminated, which generally in insects can occur spontaneously or through decreases in 'diapause intensity' via gradual changes in response to the diapause maintaining stimuli (Tauber and Tauber, 1976). In the field, diapause has often terminated for many insects during January. However, low ambient temperatures, below their developmental threshold, prevents post-diapause development from occurring as they remain in a quiescence (Denlinger, 2002).

Observations of sexually-produced *A. pisum* embryos have provided evidence of slow continuous morphological development during diapause development, suggesting that diapause is maintained in aphids by strict regulation of development rate, possibly through an inhibitory factor (Shingleton, *et al.*, 2003). The study demonstrated that the early stages of diapause up until the katatrepsis stage (where the embryo re-orientates in the egg) were temperature-independent with no differences between those embryos kept at 4 ºC days and 0 ºC nights (13L:11D)

compared with those held constantly at 10ºC (13L:11D). However, after reaching katatrepsis, diapause development becomes temperature-dependent, with the embryos held at 10ºC completing diapause first.

Studies investigating diapause development in aphids have shown that temperature plays a significant role in influencing the time from egg deposition to hatching, hatching success and, for some species, diapause termination. For example, Wipperfűrth & Mittler (1986) found that when they kept eggs of *S. graminum* at 16ºC in complete darkness, only 19% of the eggs hatched, with a development time of approximately 81.1 days. However, hatching success increased to 45% and development time was reduced to 71 days when the eggs were transferred, after 10 days, from 16ºC to 6ºC, where they were kept for a further 40 days. Similarly, two host races of *Aphis spiraecole* displayed low hatch rates at 16ºC but, following a cold treatment, hatch rates increased, and continued to increase as the cold treatment was prolonged (Komazaki, 1998). Other studies have also shown a beneficial effect of chilling on successful egg hatch and the development time of various species of aphid (Puterka and Slosser, 1986; Newton and Dixon, 1987; Via, 1992; Wang and Furuta, 2002).

While some species, like *S. graminum,* can hatch without chilling, others including *R. padi* and *S. avenae* require chilling to terminate diapause i.e. before the eggs can respond to suitable hatching conditions and begin post-diapause development (Hand, 1983; Lushai, *et al.*, 1996). This indicates that the requirement of chilling for diapause development and successful hatching varies between aphid species.

A number of researchers have estimated the thermal requirements and developmental thresholds for the egg stage of various species of aphid by exposing eggs to different constant temperatures, following a suitable period of chilling thought to terminate diapause, and measuring their development time (Wang and Furuta, 2002). For example, *Dysaphis plantaginea* (rosy apple aphid) has a lower developmental threshold of 4ºC and requires 140 day-degrees above this temperature before egg hatch will occur (Graf, *et al.*, 2006). Furthermore, such estimates appear to explain field observations, which is not unexpected, as other studies have shown that ‘temperature’ explains the differences between yearly egg hatch patterns (Dixon, 1976).

While the point at which diapause is terminated in the winter is unknown for many species, it can be determined easily through periodic sampling of eggs from the field and subsequent testing of their responses to egg-hatching conditions (Tauber and Tauber, 1976). As discussed, the ability of some aphid species to hatch in the absence of low temperatures and in complete darkness indicates that some other stimulus, apart from temperature and photoperiod, may terminate diapause. However, studies on natural insect populations have failed to identify any specific stimuli such as long day lengths, temperature or a period of chilling (Tauber and Tauber, 1976).

A limited number of studies have considered the effects of photoperiod on diapause development. A study by Wipperfűrth & Mittler (1986) which considered both temperature and photoperiod, showed that when a 12L:12D light regime was introduced following a period of chilling in dark conditions, hatching success improved marginally and development time was reduced. Furthermore, when *Rhopalosiphum insertum* eggs were collected from the field, hatching success was increased under a 16L:8D regime compared with eggs kept in the dark during incubation (James and Luff, 1982). These studies suggest, therefore, that photoperiod, in addition to temperature, could play a role.

Understanding the effects of temperature and photoperiod on the winter stages of *N. ribisnigri* will provide valuable information towards the development of a forecast to predict egg hatch in the spring. The present study aims to determine for *N. ribisnigri* (WT4850a) the requirements for sexual morph production, chilling and egg hatch in both the field and in a laboratory environment. The techniques developed can then be used in future studies on overwintering.

Methods

Experiment 4.1 Obtaining and monitoring eggs from the field

Year 2010- To enable monitoring and collection of *N. ribisnigri* eggs laid under natural field conditions, blackcurrant bushes (*R. nigrum*, See Chapter 2 General Techniques) and infested lettuce plants were caged together, using one of two types of enclosure. During July 2010, seven enclosures were erected at Warwick Crop

Centre, Wellesbourne in a field known as Long Meadow Centre (National Grid reference SP 27146 56846). Each enclosure consisted of four metal frames (each 40-50cm wide x 70-80cm high) which were covered with insect-proof netting (Enviromesh® 1.35mm mesh size to exclude aphids) as shown in Figure 4.1.1a. Three blackcurrant bushes were then planted within the enclosure and MyPex® was laid over the soil to suppress weed growth.

At the same time, two walk-in cages (3m long x 2m wide x 2m high) were also placed in Long Meadow Centre. These contained three blackcurrant bushes as shown in Figure 4.1.1b. This method has been successfully used previously by Collier (2007a).

a) 

b) 

c) 

Figure 4.1.1 a-c **a)** Seven enclosures covered with insect proof netting **b)** Walk-in cages **c)** Plastic planting troughs containing lettuce plants, all used to monitor and collect *N. ribisnigri* eggs laid under natural field conditions.

On 18 August 2010, 80 seeds of lettuce cv. Saladin were sown in vermiculite and were transplanted individually into 400ml square plant pots one week later, where they were then grown for a further three weeks.

On 15 September 2010, the lettuce plants were infested with *N. ribisnigri* (clone WT4850a) by inoculating each plant with 20 aphids, consisting of a mixture of

developmental stages. These were transplanted into the enclosures. Six lettuce plants were planted through the MyPex® in each of the seven enclosures and the remainder were divided between the two large cages. Fresh infested lettuce plants were added to the enclosures as required and irrigation was applied using a sprinkler system.

Once planting had taken place, the blackcurrant bushes in each enclosure were monitored weekly for the arrival of sexual morphs and the deposition of eggs. The small enclosures were numbered 1-7 and each of the three small blackcurrant bushes was monitored. In the two larger walk-in cages, six branches of each of the three blackcurrant bushes in each cage were tagged and these were checked each week. Due to other experimental commitments, sampling did not commence until 1 November 2010 and continued until 6 December 2010. When necessary, specimens of the aphids were collected and taken to the laboratory for identification. Weekly monitoring for egg hatch commenced on 6 January 2011 and continued until fundatrices emerged.

Year 2011- During 2011, the method used in 2010 was repeated but the use of the large cages was abandoned as during monitoring in 2010, *H. lactucae* was found depositing eggs on the blackcurrants inside the cages, indicating that they were not excluding other aphid species. Furthermore, once eggs had been laid it was impossible to identify them until they had hatched in the following spring.

The plant raising and infestation process was repeated for the seven enclosures as per 2010. Plants were sown on 13 June 2011 and were infested with *N. ribisnigri* and planted on 13 July 2011. Instead of planting infested lettuce plants through the MyPex®, six lettuce plants were planted into plastic trough planters, which were then placed inside the enclosures as shown in Figure 4.1.1c. New troughs were added as required and irrigation was applied using sprinklers.

Weekly monitoring for sexual morphs and egg deposition commenced on 5 September 2011 until 17 December 2011. Weekly monitoring for egg hatch commenced on 6 January 2012 until fundatrices emerged. The small enclosures were numbered 1-7 and each of the three small blackcurrant bushes was monitored. When necessary, specimens of the aphids were collected and taken to the laboratory for identification.

Experiment 4.2 Induction of sexual morphs

To induce sexual morph production, the natural conditions that *N. ribisnigri* would experience in September were determined. Using the 2009 records from the University of Warwick, Wellesbourne meteorological station, the mean maximum and minimum temperatures for September were calculated, which gave a mean maximum temperature of 19.3°C and a minimum of 10°C. These values gave a mean of 14.7°C, which was the temperature used in the experiment. The 'typical' photoperiod for September (13L:11D) was also determined using the daylight hours recorded in Coventry during 2009 (Timeanddate, 2010).

The effect on sexual morph production of a lower temperature (12°C) and longer photoperiods (14L:10D, 16L:8D) was also investigated. The longer photoperiods were included to determine which day lengths in excess of 13 hours were suitable for inducing sexual morph production following confirmation that 13L:11D induced sexual morphs.

The conditions used in this experiment are shown in Table 4.2.1, the photoperiod of 16L:8D was not paired with 15°C as this condition was used in other experiments (Chapter 3) and did not result in production of any sexual morphs.

Table 4.2.1 Rearing regimes (temperature (°C) and photoperiod) and the experiment start dates to determine the conditions required for the induction of sexual morphs.

Treatment num.	Temperature (°C)	Photoperiod	Experiment start date
1	12	16L:8D	27 February 2012
2	15	14L:10D	13 May 2012
3	15	13L:11D	24 March 2011
4	12	13L:11D	26 October 2011
5	12	14L:10D	8 May 2012

Samples of *N. ribisnigri* were exposed to each of the conditions shown in Table 4.2.1 using the following method, which was adapted from methods used by Lamb and Pointing (1972) and MacKay (1987).

Initially ten third instar nymphs (G_0) of WT *N. ribisnigri* (clone 4850a) were obtained from the stock culture. In later experiments, 15 aphids were used to compensate for any mortality and the reduced reproduction at the lower treatment temperature. The aphids were then grown on lettuce plants (cv. Saladin) under one of the five treatment regimes until they began to reproduce (G_0), which provided pre-natal conditioning of the embryos. The lettuce plants used were at BBCH growth stage 14 (4^{th} true leaf unfolded).

The G_0 aphids were then moved to new lettuce plants (growth stage 14) and left for either 24 hours or 48 hours under the same treatment regime to provide G_1 nymphs of a similar age. Aphids kept at 15°C were left for 24 hours and those at 12°C for 48 hours because reproduction was slower at the lower temperature. Aphids were divided between several plants to avoid the development of alate parthenogenetic aphids as a result of crowding.

The G_0 aphids were then discarded and the G_1 nymphs were left to develop to adulthood under the treatment regime used to provide post-natal conditioning. The nymphs were divided between two lettuce plants (cv. Saladin, BBCH growth stage 14) to avoid a crowding stimulus. Once the G_1 nymphs reached adulthood they were transferred to individual lettuce plants and kept under the same treatment regime, where they began to produce G_2 offspring.

At 15°C, on days 2 and 4, and every three days thereafter, the G_1 adults were moved to a new plant (BBCH growth stage 14). The G_2 nymphs were allowed to remain on the natal plant to develop to adulthood. The type of adult morph was then recorded. At 12°C, G_1 adults were moved to a new plant every four days as their development and reproduction were slower. This method provided batches of offspring from the reproductive sequence.

The morphs produced were apterous parthenogenetic females, alate parthenogenetic females, males or gynoparae. Males were easily identified by their genitalia as shown in Figure 4.2.2, but gynoparae and alate parthenogenetic females were very

similar in appearance. Therefore, to distinguish between these two morphs, the alates were kept on a lettuce plant and if nymphs were produced they were identified as parthenogenetic females and if no nymphs were produced they were identified as gynoparae. This approach made the assumption that gynoparae were only able to produce oviparae on blackcurrant.

When a treatment regime resulted in production of sexual morphs, Experiment 4.3 was undertaken to confirm the type of sexual morph produced, as the production of eggs would confirm the production of males and gynoparae.

Figure 4.2.2 Male *N. ribisnigri* (left) and alate parthenogenetic female (right).

Experiment 4.3 Obtaining eggs in the laboratory

Preliminary experiment- Once Experiment 4.2 had identified conditions which stimulated production of sexual morphs, aphids were kept under these conditions for several generations to see if eggs were produced.

During April 2011, Experiment 4.2 indicated that rearing conditions of 15°C with 13L:11D stimulated the production of males. Therefore, on 20 July 2011, blackcurrant cuttings were paired with infested lettuce plants (cv. Saladin, BBCH growth stage 14) (*N. ribisnigri* (clone 4850a), as shown in Figure 4.3.1a, and placed in an incubator at 15°C with 13L:11D to see whether the production of gynoparae

and males was stimulated. The pairs of blackcurrant cuttings and lettuce plants were enclosed using a micro-perforated polypropylene bag to confine the aphids.

Once the males and gynoparae had been produced it was thought that they would move onto the blackcurrant cuttings and continue the rest of the winter lifecycle. By 24 September 2011 no eggs had been deposited, so the temperature was lowered to 12°C which reflected the 'October' temperature. Once eggs had been produced they were transferred outside to experience natural conditions to see if the eggs were 'viable' (no visible signs of dessication or fungal growth) and would hatch during the following spring.

This method was also adapted later for small blackcurrant plants where the infested lettuce plants were transplanted into the soil around the base of each small blackcurrant plant as shown in Figure 4.3.1b. Prior to use, the blackcurrant cuttings and plants were washed to remove all other insects.

Main experiment- Following successful production of eggs from the preliminary experiment, four blackcurrant plants were prepared and the experiment was repeated on 25 November 2011. Aphids were kept at 15°C with 13L:11D for 56 days and then the temperature was reduced to 12°C on 20 January 2012. The production of eggs was also screened at 15°C 13L:11D only for a longer period of time (63 days), which was initiated on 28 September 2012.

To determine whether the temperature change from 15 to 12°C was necessary, or whether being kept continuously 12°C could induce egg production, a new blackcurrant plant was prepared on 8 June 2012 and placed in an incubator at 12°C with 13L:11D.

Other regimes which were screened for induction of egg production included 15°C with 14L:10D which was initiated in an incubator on 28 September 2012 and 12°C 14L:10D which was initiated on 27 July 2012 in CE Room 5. Table 4.3.2 summarises the experiments performed.

a)  **b)** 

Figure 4.3.1 a-b **a)** Blackcurrant cuttings paired with infested lettuce **b)** Small blackcurrant bushes paired with infested lettuce to obtain eggs under conditions inducing production of sexual morphs.

Table 4.3.2 Summary of the rearing regimes, the number of replicates and the start dates for experiments to obtain eggs in the laboratory.

Rearing regime	**Replicate number**	**Experiment start date**
15°C 13L:11D transferred to 12°C 13L:11D	1	20 July 2011
	2	25 November 2011
15°C 13L:11D	1	28 September 2012
12°C 13L:11D	1	8 June 2012
15°C 14L:10D	1	28 September 2012
12°C 14L:10D	1	27 July 2012

Experiment 4.4 The development and host plants of the fundatrix

A preliminary experiment was carried out using the fundatrices which emerged from the eggs obtained from Experiment 4.3 (preliminary experiment) to investigate the developmental time and suitable host plants of the fundatrix.

Fundatrices were observed hatching from the eggs on 13 February 2012. On 8 March 2012, several of the fundatrices were collected and placed on leafy blackcurrant

cutting at 12 and 16°C 16L:8D (CE Room 5 and Sanyo incubator respectively) to determine how long it took them to reach adulthood.

On 13 March 2012 five more fundatrices were placed on lettuce plants (cv. Pinokio) which produced alate offspring. Some of the alate offspring were transferred to a blackcurrant plant where they were confined on a leaf using micro-perforated polypropylene bag (200mm x 500mm) (Figure 4.4.1). Eight alate/apterous parthenogenetic *N. ribisnigri* from the WT4850a cultures were also confined to a blackcurrant leaf.

Figure 4.4.1 Experimental set-up used to enclose alate offspring produced by fundatrices on lettuce and alate/apterous parthenogenetic *N. ribisnigri* from the 4850a culture on the leaves of a blackcurrant plant.

Experiment 4.5 Diapause termination

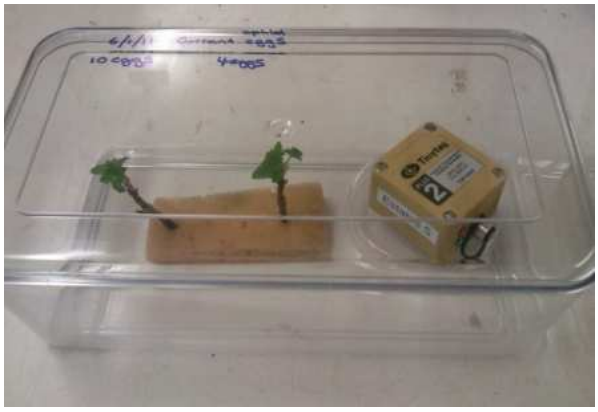
Preliminary experiment Year 2011-A preliminary experiment was conducted using the small number of eggs (approximately 60 eggs) obtained in the field from Experiment 4.1 during 2010. Commencing on 6 January 2011, approximately ten eggs were sampled by taking cuttings from several of the blackcurrant bushes

contained in enclosure 1-7 located in Long Meadow Centre. Samples were also taken on 20 January, 1, 17 and 25 February and 4 March 2011. Eggs were only sampled from the seven small enclosures due to the occurrence of *H. lactucae* in the large cages during the period of egg deposition.

Sampled cuttings were stood in a piece of domestic foamed plastic polymer sponge in a lidded container (15 x 30 cm) and transferred to an incubator at 16°C 16L:8D as shown in Figure 4.5.1a. A preliminary trial on *N. ribisnigri* showed that these conditions induced egg hatch (Collier, 2007a). The eggs were checked at approximately 2-day intervals to see if they had hatched.

The cuttings were watered twice a week by adding water to the container, which the sponge absorbed. A closed system ensured high humidity to avoid egg desiccation. Tiny Tag® loggers were placed inside the container to record humidity and temperature.

a)



b)

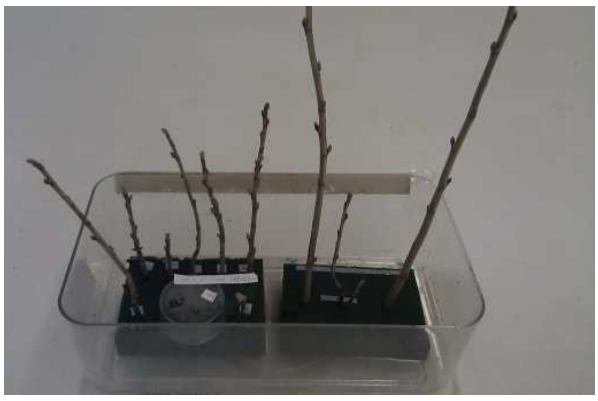


Figure 4.5.1a-b **a)** The method used during 2011 to hatch field-sampled eggs in an incubator **b)** The method used during 2012 to hatch field-sampled and laboratory-produced eggs in an incubator.

Main experiment 2012- Eggs produced during 2011 under natural field conditions in Experiment 4.1 were used. Eggs were also obtained from the laboratory, by transferring the sexual morphs produced during the preliminary experiment of Experiment 4.3 to four newly-prepared blackcurrant plants on 2 November 2011. On 8 November 2011, the four blackcurrant plants supporting the sexual morphs (which had commenced depositing eggs) were moved outside to expose them to natural conditions comparable with those experienced by the field-produced eggs. The blackcurrant plants were covered with micro-perforated polypropylene bags to protect the sexual morphs from predators and were left outside for more eggs to be deposited.

Once the eggs had been obtained, sampling of eggs from the seven field enclosures, and of eggs produced in the laboratory, began on 26 November 2011 with approximately 30 eggs being removed from each location. Samples were also taken on 9 and 16 December 2011, 6, 20 and 30 January and 13 and 24 February 2012.

Sampled cuttings were stood in Oasis® floral foam which was then placed into a container (7.5 x 15 cm) and transferred to an incubator at 16°C 16L:8D as shown in Figure 4.5.1b. On average, eggs were checked three times a week to see if they had hatched. Eggs which were infected with fungus or had desiccated were removed. The cuttings were watered twice a week by adding water to the container, which the Oasis® absorbed. The container could not be sealed with a lid as the cuttings were too tall.

Experiment 4.6 Egg chilling requirements and thermal requirements for hatching

Preliminary experiment- A preliminary experiment was carried out to determine the best methods for determining the chilling requirements of *N. ribisnigri* eggs. Eggs were obtained on four blackcurrant plants from Experiment 4.3 (second repeat of the temperature transfer experiment from 15°C transfer to 12°C 13L:11D started on 25 November 2011), which provided eggs by 2 February 2012.

On 15 February 2012, three of the small blackcurrant bushes were removed from the incubator and all of the leaves were removed, together with the sexual morphs. Each

small blackcurrant bush was covered with a labelled brown paper bag as shown in Figure 4.6.1a and a single blackcurrant bush was then exposed to each of three treatment regimes i.e. 0, 5 or 10°C D:D. The experimental conditions were provided by an incubator (0°C) a cold store (5°C) and CE Room 1 in the IRU (10°C). Environments were kept in complete darkness. A Tiny Tag® logger was placed in each environment to monitor the temperature and humidity.

On 24 February 2012, egg sampling commenced. Each week, approximately 20 eggs were removed from each treatment regime by taking cuttings, which were then labelled with the sampling date and treatment temperature. Sampling continued until no eggs remained. The cuttings were stood in Oasis® floral foam held in a container (7.5 x 15 cm) of water and transferred to an incubator at 16°C 16L:8D. Two to three times a week the eggs were checked to see if they had hatched and eggs which were infected with fungus or had desiccated were removed. A closed system was not used as the cuttings were too tall to be covered with a lid to maintain a high humidity

a)  **b)** 

Figure 4.6.1 a-b **a)** Potted small blackcurrant bushes covered with brown paper bags **b)** Cuttings stood in Oasis® floral foam.

Main experiment -On 6 March 2012, four small blackcurrant bushes were prepared to acquire eggs as per the method described in Experiment 4.3 (at 15°C 13L:11D (preliminary experiment). By 5 April, no eggs had been produced and the temperature was lowered to 12°C. On 12 May, oviparae and males were observed copulating and on 25 May, eggs were found on three of the four small blackcurrant bushes.

On 8 June 2012, once the number of the eggs had increased, the blackcurrant bushes were removed from the incubator and the branches of the small bushes were cut to

provide twelve cuttings supporting various numbers of eggs. All the leaves were removed, together with the sexual morphs.

Six cuttings, supporting 303 eggs, were placed in an incubator at 0ºC D:D while the remaining cuttings, supporting 297 eggs, were placed in an incubator at -5ºC D:D. Each cutting was placed in a re-sealable zipped plastic bag to maintain high humidity. A Tiny Tag® logger was placed in one of the bags at each temperature to monitor the temperature and humidity

On 28 June 2012, egg sampling began and this continued until egg hatch occurred. Each week, approximately 25 eggs were removed from each incubator by taking cuttings. The cuttings were stood in Oasis® floral foam, labelled with the sampling date and temperature, and placed in a container which was transferred to an incubator at 16°C 16L:8D as shown in Figure 4.6.1b. A Tiny Tag® logger was placed in the incubator to monitor the temperature and humidity.

Each week, the eggs were checked to see if they had hatched and eggs which were infected with fungus or had desiccated were removed. The cuttings were too large to use a closed system to maintain high humidity, so a large tray of water was placed in the incubator and refilled regularly to try and raise the humidity.

Results

Experiment 4.1 Obtaining and monitoring eggs from the field

Year 2010- When monitoring of the blackcurrant bushes began in November 2010, several aphid eggs had already been deposited in both the large cages and small enclosures. The eggs had been deposited mainly in the angle between a stem and a bud and were initially green in colour, before turning black. The eggs which remained green were unfertilised (Leather, 1980).

Unfortunately, individuals of a species of aphid other than *N. ribisnigri* were observed depositing eggs on the blackcurrant bushes in one of the large cages. Specimens were collected and sent to Rothamsted Research where they were identified as *H. lactucae.* As a result, the large cages were no longer monitored as it

was impossible to distinguish between eggs of *N. ribisnigri* and *H. lactucae* until they hatched in the following spring.

Weekly monitoring of the blackcurrant bushes to record egg hatch began on 6 January 2011, and on 17 February, 20 nymphs were observed on a blackcurrant bush inside Enclosure 2.

Year 2011- In 2011, monitoring for sexual morphs and eggs began earlier, on 5 September 2011. Of the seven enclosures set up, only five had *N. ribisnigri* remaining on the lettuce plants. On 7 October, the first alate *N. ribisnigri* were observed on the blackcurrant bushes in the enclosures and these were assumed to be gynoparae as they were not males. As there were no oviparae nymphs present, they could, however, have been alate parthenogenetic aphids. An unknown aphid was also present in high numbers on the blackcurrant bushes and on the netting of Enclosures 1 and 2. Specimens of the aphid were collected and sent to Rothamsted Research where they were identified as *Eriosoma ulmi*. Fortunately, this aphid does not lay eggs on *Ribes* species and was migrating back to its winter host (elm) for the winter.

On 14 October, alate *N. ribisnigri* were observed producing nymphs and were therefore assumed to be gynoparae. On 21 October 2011, *N. ribisnigri* males and gynoparae were observed on the blackcurrant bushes. Following this, the first eggs were observed on 28 October, with one egg in Enclosure 4 and two eggs in Enclosure 5, both of which were accompanied by an ovipara. By 11 November, four of the five enclosures contained eggs and many gynoparae and oviparae were depositing nymphs and eggs. On 5 December, approximately 320 eggs were observed on the blackcurrant bushes present in each enclosure (3 bushes per enclosure), with Enclosure 5 containing the most eggs (Figure 4.1.2). At this time, large numbers of oviparae were still present on the blackcurrant bushes.

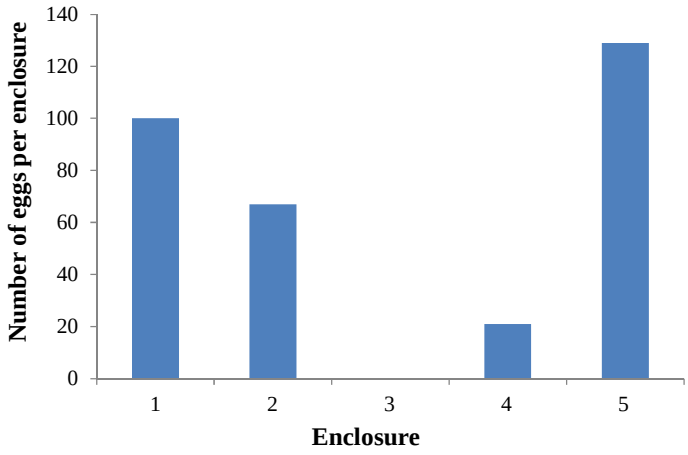


Figure 4.1.2 Number of eggs on blackcurrant bushes in each enclosure.

On 6 January 2012, monitoring for egg hatch began. Nymphs were first observed on 24 February and these had hatched since the previous sampling date of 17 February. The buds of the blackcurrant bushes had not started to open by the time the first nymphs hatched and the fundatrices died. It was not until 8 March that the buds were opening. At this time, three nymphs were observed in Enclosure 1 and one in Enclosure 5. The nymphs were, however, not present on subsequent monitoring dates. No fundatrices had established on the blackcurrant bushes by 29 March.

Experiment 4.2 Induction of sexual morphs

Following pre-natal and post-natal conditioning under a range of temperatures and photoperiod regimes, adult morphs were recorded as shown in Table 4.2.3 a-e. The only treatment regime which did not lead to production of a sexual morph was 12°C 16L:8D where only apterous and parthenogenetic alate *N. ribisnigri* were observed. Apterous forms were common to all treatments and occurred in the same batches of offspring as gynoparae (Table 4.2.3d and e) and males (Table 4.2.3a, b and d).

Males occurred at 15°C 14L:10D, 15°C 13L:11D and 12°C 14L:10D where they were observed from Batches 3, 4 and 2 respectively. Parthenogenetic alate *N. ribisnigri* occurred in the same batches as the males.

Gynoparae occurred under two rearing regimes. As Table 4.2.3e and Figure 4.2.4 illustrate, a considerable number of gynoparae were produced from Batch 1 through to Batch 7 at 12°C 13L:11D, while no males were observed. Conditioning at 12°C 14L:10D (Table 4.2.3d) also resulted in the production of gynoparae in Batches 1 and 2 but no more than three individuals were observed. Males were also produced in Batch 2. Neither of the regimes at 15°C produced gynoparae, but they did produce males.

As different numbers of aphids were used to produce G_0 offspring for each treatment regime, the differences between each treatment in the numbers produced per batch were not meaningful. Also, the alates which occurred at the rearing regime 15°C 13L:11D were not screened to see if they produced nymphs on lettuce and therefore were not determined as gynoparae or parthenogenetic alates in this experiment.

Table 4.2.3a-e Percentage (%), total (T) and numbers of apterae, alate, gynoparae (Gynop) and male aphids produced during the reproductive life of *N. ribisnigri* adults kept at a) 15°C 14L:10D, b) 15°C 13L:11D c) 12°C 16L:8D d) 12°C 14L:10D and e) 12°C 13L:11D.

a) 15°C 14L:10D	**Batch number**													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**T**	**%**
Apterae	102	69	56	88	49	55	48	14	9	2	0	5	497	75
Alates	2	4	2	8	10	9	8	4	2	0	0	4	53	8
Gynop*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Males	0	0	8	26	23	10	12	13	11	8	4	0	115	17

**Alates were screened to see if they produced nymphs on lettuce*

b) 15°C 13L:11D	**Batch number**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**T**	**%**
Apterae	55	76	72	62	20	3	10	298	84
Alates	3	0	1	7	2	0	1	14	4
Gynop*	0	0	0	0	0	0	0	0	0
Males	0	0	0	10	17	7	8	42	12

**Alates were not screened on lettuce to determine if they produced nymphs on lettuce*

c) 12°C 16L:8D	**Batch number**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**T**	**%**
Apterae	16	2	1	5	3	0	2	0	29	14
Alates	36	46	48	27	13	10	1	2	183	86
Gynop*	0	0	0	0	0	0	0	0	0	0
Males	0	0	0	0	0	0	0	0	0	0

**Alates were screened to see if they produced nymphs on lettuce.*

d) 12°C 14L:10D	**Batch number**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**T**	**%**
Apterae	74	33	35	27	42	23	3	0	3	5	0	245	57
Alates	5	2	12	20	6	2	0	0	2	2	0	51	12
Gynop*	2	1	0	0	0	0	0	0	0	0	0	3	0.7
Males	0	5	31	18	28	26	13	1	4	4	1	131	30

**Alates were screened to see if they produced nymphs on lettuce*

e) 12°C 13L:11D	**Batch number**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**T**	**%**
Apterae	7	6	1	0	0	0	1	0	2	17	30
Alates	0	0	0	0	0	0	0	0	0	0	0
Gynop*	6	13	4	7	4	3	2	0	0	39	70
Males	0	0	0	0	0	0	0	0	0	0	0

**Alates were screened to see if they produced nymphs on lettuce*

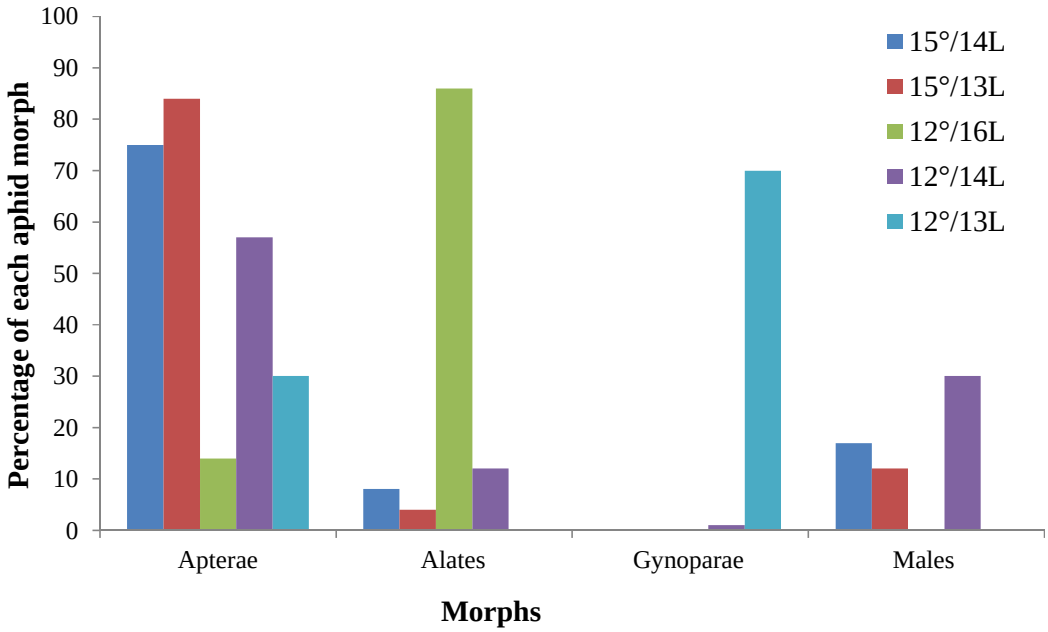


Figure 4.2.4 Percentage morphs produced under each rearing regime.

Experiment 4.3 Obtaining eggs in the laboratory

Preliminary experiment- On 25 October 2011, eggs were obtained by exposing blackcurrant cuttings paired with lettuce plants infested with *N. ribisnigri* to 15°C 13L:11D from 20 July 2011 and then to 12°C 13L:11D from 24 September 2011. However, it was unclear whether it was the 31 days at 12°C from 24 September to 25 October which resulted in the production of eggs or the change from 15°C to 12°C,

as no eggs had been produced prior to the temperature change. Figure 4.3.3a-c shows the blackcurrant cutting supporting eggs deposited during the experiment, which were moved outside to experience natural conditions to see if the eggs were 'viable' and would hatch during the following spring in Experiment 4.5 (diapause termination).

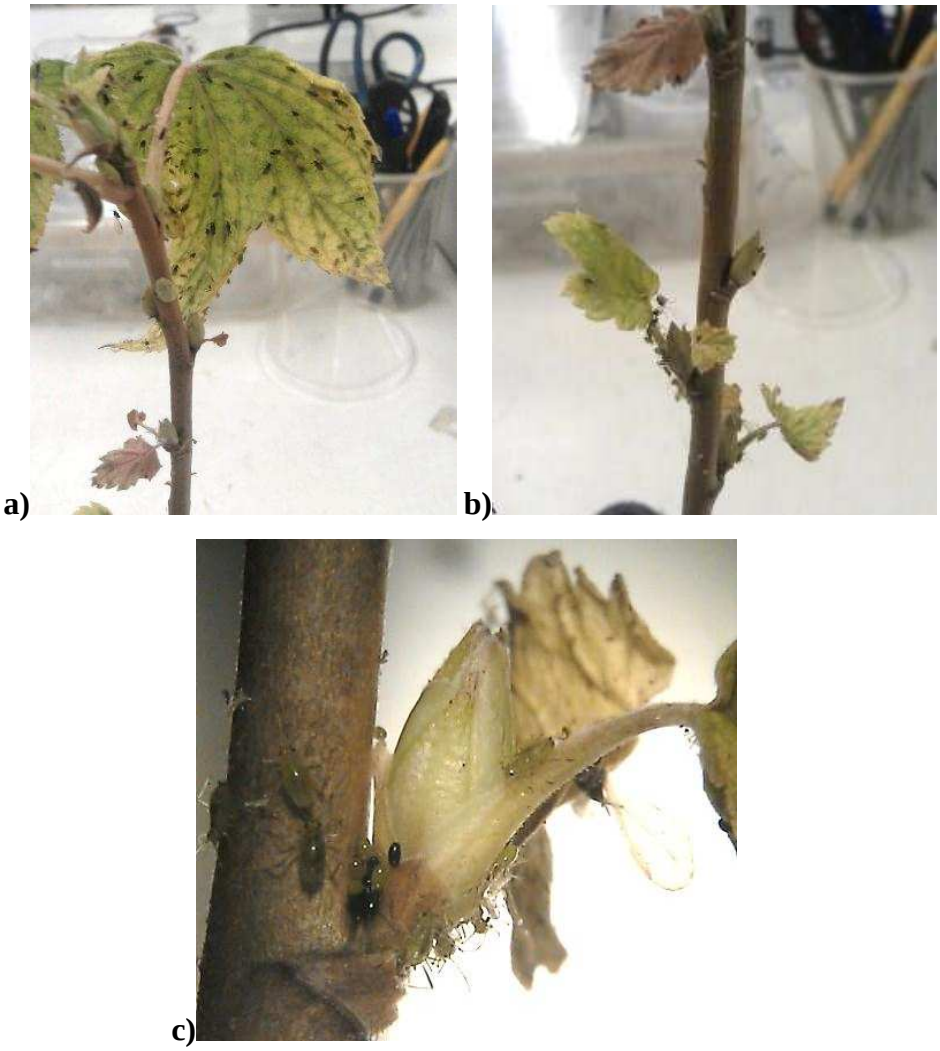


Figure 4.3.3 a-c **a)** Gynoparae, oviparae and males on blackcurrant leaf **b)** Sexual morphs and eggs **c)** Oviparae and eggs.

Main experiment- On 25 November 2011 four potted blackcurrant plants were prepared and the preliminary experiment was repeated. On 20 January 2012, when the temperature was reduced to 12 °C, two eggs were observed on a blackcurrant bush. On 2 February 2012, following the temperature decrease, considerably more eggs were observed on three of the four blackcurrant plants. Thus, whilst a few eggs

were produced by Day 56, egg numbers had increased considerably by day 69, once the temperature had been lowered.

As the preliminary experiment suggested that 15°C 13L:11D alone did not result in egg production, while the second repeat did (although only a low number of eggs were observed), this condition was tested without the temperature change to 12°C. This showed that after 63 days at 15°C 13L:11D no eggs were produced.

On 8 June 2012 a small blackcurrant bush was placed at 12°C 13L:11D to see if this regime alone would result in the production of eggs. By 27 July, several eggs had been produced and more eggs were observed on 2 August. Thus eggs were produced between 41-49 days. Unfortunately, it was not possible to determine whether eggs were produced at 12°C 14L:10D, as when infested lettuce plants were paired with a small blackcurrant bush, an individual aphid, which was not *N. ribisnigri*, was found to be laying eggs. This aphid must have been missed when the blackcurrant was prepared and extraneous insects were removed. Table 4.3.4 summarises the outcomes from Experiment 4.3.

Table 4.3.4 Outcome of the rearing regimes used to screen for the induction of egg production when lettuce plants infested with *N. ribisnigri* were paired with small blackcurrant bushes or cuttings.

Temperature (°C)	**Photoperiod**	**Eggs produced**	**Days exposed to regime and in some cases when eggs were produced**
15	14L:10D	No	63
15 transferred to 12	13L:11D	No Yes	66 at 15°C 31 at 12°C
15 transferred to 12	13L:11D	Yes Yes	56 at 15°C 13 at 12°C
15	13L:11D	No	63
12	14L:10D	Un-determined	
12	13L:11D	Yes	49

Experiment 4.4 The development and host plants of the fundatrix

When the fundatrices were placed onto blackcurrant leaves at 12 and 16°C 16L:8D, the leaves curled quickly and the fundatrices died, meaning that development time to adulthood could not be determined.

On 24 March 2012 the fundatrices placed on a lettuce plant developed to adulthood and had produced alate offspring, which were themselves producing nymphs.

On 11 April 2012, the four alates produced by the fundatrices and the eight alate/apterous parthenogenetic *N. ribisnigri* from the culture placed on a currant leaf were dead and had not produced any offspring. The alates produced by the fundatrices which were left on the lettuce plant continued to develop and produce offspring.

Experiment 4.5 Diapause termination

Preliminary experiment **–** To determine when diapause had ended naturally in the field, sampling of the small number of eggs deposited in the field in 2010 was carried out approximately every two weeks which began on 6 January 2011. Table 4.5.2 shows the numbers of eggs which had hatched by each monitoring date for eggs which has been sampled over six dates.

On 9 February 2011 the first nymph was observed on a cutting which had been sampled from the field enclosures on 1 February, thus taking approximately eight days to hatch (Figure 4.5.3). By the end of the monitoring period, two nymphs out of 13 eggs had hatched on this cutting.

On 21 February 2011, one nymph was observed on one of the cuttings which were sampled on 17 February, taking approximately four days to hatch. Following this, a total of four nymphs hatched on this cutting out of the 14 eggs sampled.

When cuttings were sampled from the field on 25 February 2011, one nymph was already present. Following transfer to 16°C 16L:8D, a further nymph was observed on 1 March, hatching within two days. Two of the four eggs on this cutting hatched.

On the final sampling date in March, three eggs hatched three days after being transferred 16°C 16L:8D. The highest number of eggs which hatched were sampled from the field on 17 February.

Egg mortality was relatively high and appeared to be due to desiccation and fungal infection, particularly when eggs were at the base of the cuttings and close to the sponge. Tinytag® recordings indicated that the mean temperature in the containers held at 16°C 16L:8D was 16.64°C. Recordings of humidity were very variable between 6 January and 1 February 2011 and following replacement of the logger the rest of the experiment was exposed to a relative humidity with a mean value of 73.42%.

Table 4.5.2 Number of field-produced eggs which hatched following sampling on six occasions. The total number of eggs sampled is also shown.

	Sampling date					
	6/1/11	**20/1/11**	**1/2/11**	**17/2/11**	**25/2/11**	**4/3/11**
Monitoring date						
10/1/11	0					
11/1/11	0					
12/1/11	0					
13/1/11	0					
14/1/11	0					
17/1/11	0					
18/1/11	0					
19/1/11	0					
20/1/11	0					
24/1/11	0	0				
25/1/11	0	0				
26/1/11	0	0				
27/1/11	0	0				
30/1/11	0	0				

1/2/11	0	0				
6/2/11	0	0	0			
7/2/11	0	0	0			
8/2/11	0	0	0			
9/2/11	0	0	1			
10/2/11	0	0	0			
11/2/11	0	0	0			
14/2/11	0	0	0			
15/2/11	0	0	0			
21/2/11	0	0	1	1		
22/2/11	0	0	0	1		
23/2/11	0	0	0	0		
25/2/11	0	0	0	1		
28/2/11	0	0	0	0	1	
1/3/11	0	0	0	0	1	
2/3/11	0	0	0	1	0	
3/3/11	0	0	0	0	0	
4/3/11	0	0	0	0	0	0
7/3/11	0	0	0	0	0	3
Total hatched	0	0	2	4	2	3
Total eggs sampled	14	10	13	14	4	4

Figure 4.5.3 Newly-hatched *N. ribisnigri* fundatrix.

Main experiment- Sampling began on 26 November 2011. Table 4.5.4 shows the number of eggs which hatched for eggs sampled on five sampling dates. Eggs were produced under field conditions (Field) or in the laboratory and then transferred to the field (Laboratory).

On transferring the eggs to 16°C 16L:8D, the first fundatrix hatched by 23 January 2012, having being sampled from eggs produced in the laboratory on 6 January 2012. From this sampling date onwards, at least one fundatrix hatched from each sample of eggs, whether produced in the field or the laboratory. After 13 February, all the laboratory-produced eggs had been used and only field-produced eggs were sampled on 24 February.

Field-produced eggs did not commence hatching until 1 February on blackcurrant plants sampled from the field on 20 January 2012. On later sampling occasions, more field-produced eggs hatched compared with laboratory-produced eggs.

As shown in Table 4.5.4, percentage hatch was generally low. In addition, a considerable number of eggs were removed because of fungal infection or desiccation, although losses were reduced following the later sampling dates, when the eggs were not monitored for as long. Because eggs were lost, egg hatch is expressed as a percentage of the number of eggs which appeared 'viable' rather than the total sampled. The greatest proportion of eggs hatched from field-produced

samples taken on 24 February, where 45.8% of 'viable' eggs hatched by 27 February.

Table 4.5.4 Number of eggs which hatched after being sampled on five occasions. Eggs were field-produced (Field) or produced in the laboratory (Lab). The total number of eggs sampled and the number of eggs removed because of fungus infection or desiccation are also shown, as is the percentage of 'viable' eggs which hatched.

	Sampling date and egg origin								
	06/01/2012		20/01/2012		30/01/2012		13/02/2012		24/02/2012
	Field	Lab	Field	Lab	Field	Lab	Field	Lab	Field
Monitoring date									
13/1/2012	0	0							
16/1/2012	0	0							
20/1/2012	0	0							
23/1/2012	0	1	0	0					
24/1/2012	0	0	0	0					
26/1/2012	0	0	0	2					
30/1/2012	0	0	0	0					
01/2/2012	0	1	1	1	0	0			
03/2/2012	0	0	0	0	1	0			
11/2/2012	0	0	1	0	3	0			
15/2/2012	0	0	0	0	0	1			
17/2/2012	0	0	0	0	1	1	1	0	
20/2/2012	0	0	0	0	0	0	4	1	
23/2/2012	0	0	0	0	0	0	2	0	
27/2/2012	0	0	0	0	0	0	1	0	11
29/2/2012	0	0	0	0	0	0	0	0	0

Total no. hatched	0	2	2	3	5	2	8	1	11
Total no. eggs sampled	27	27	29	33	33	29	28	10	27
Total no. eggs removed	18	21	19	13	6	9	5	2	3
Percentage hatch of 'viable' eggs (%)	0	33	20	20	18.5	10	34.8	12.5	45.8

For field-produced eggs the time required for 50% of the eggs to hatch decreased as the sampling dates became later (Figure 4.5.5). For example, only approximately 2 days were required for 50% of the field-produced eggs to hatch when kept at 16°C 16L:8D following sampling on 24 February 2012. While this relationship is less distinct for laboratory-produced eggs due to the lack of data, the trend line is similar to that of the field-produced eggs.

Cumulative hatching curves for both field-produced and lab- produced eggs sampled from the field on different dates and maintained at 16°C 16L:8D are shown in Figure 4.5.6. Similarly to Figure 4.5.5 it shows that eggs collected from the field at later sampling dates compared with earlier sampling dates, required less time at 16°C 16L:8D before they hatched but also had a higher percentage of eggs hatching sooner. None of the field-produced eggs collected at the earliest sampling date (6 January) hatched when kept for 54 days at 16°C 16L:8D.

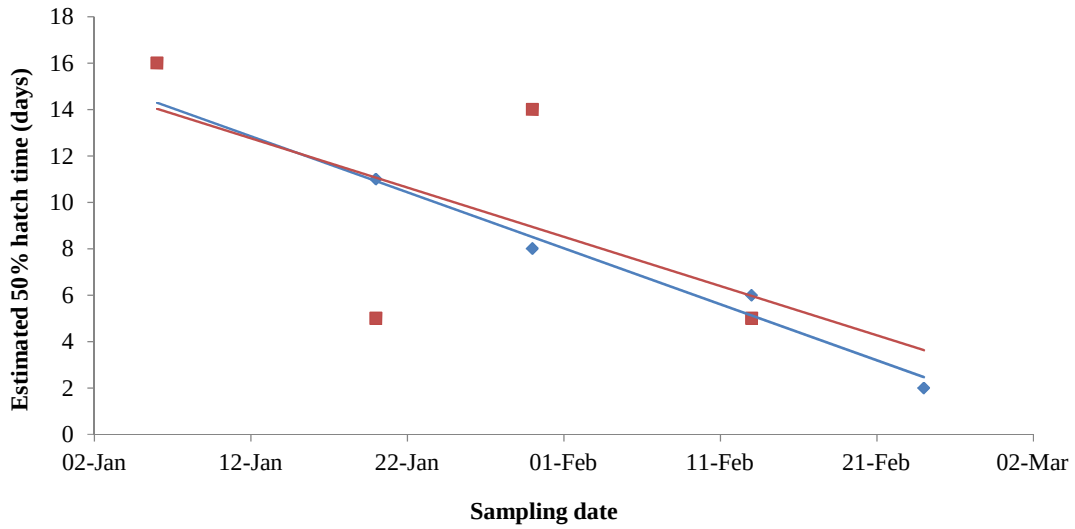


Figure 4.5.5 Estimated time (days) until fifty per cent of the field-produced (R^2 = 0.9708, Y= -0.2416 + 9901*x*) and lab-produced (R^2 = 0.3416, Y= -0.2124 + 8705.6*x*) eggs collected from the field (2012) at each sampling date hatched when kept at 16°C 16L:8D. ◆ Field-produced eggs, ■ lab-produced eggs, — Linear (field) and — Linear (Lab).

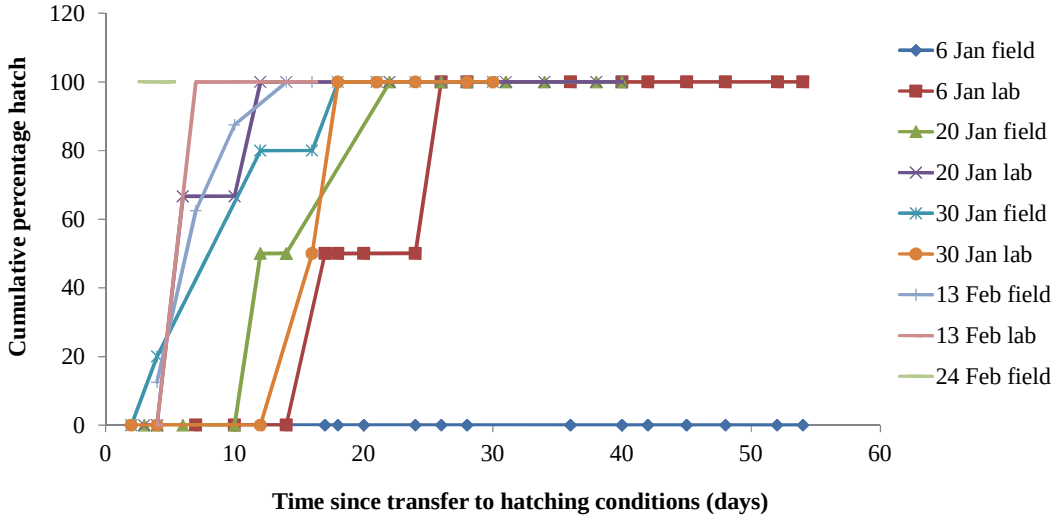


Figure 4.5.6 Percentage of fundatrices hatching from field-produced and lab-produced eggs collected from the field at each sampling date and kept at 16°C 16L:8D for up to 54 weeks (2012).

Experiment 4.6 Egg chilling requirements and thermal requirements for hatching

Preliminary experiment **–** Weekly sampling began on 24 February 2012 for eggs that were chilled at 0, 5 and 10°C. Actual mean temperatures were 0.62, 5.45 and 11.24°C respectively. Relative humidity was recorded only for the 10°C treatment, with a mean of 93.7%. Sampling from the eggs kept at 5 and 10°C continued until 23 April and until 29 May for the eggs kept at 0°C. Unfortunately none of the eggs hatched. A large amount of fungal infection was observed on the blackcurrant plants and the eggs as time progressed. Fungal growth also occurred quickly on the cuttings once they were transferred to the incubator.

Main experiment- Eggs were chilled at 0°C (actual mean -0.09°C and 89.9% RH) or -5°C (actual mean -4.64°C and 84.9% RH) from 8 June 2012 and were then sampled at weekly intervals from 28 June and placed at 16°C 16L:8D (actual mean 16.2°C and 83.8%RH). Few eggs hatched overall. On 24 August, however, a single nymph was observed on blackcurrant cuttings sampled from the 0°C D:D regime and placed at 16°C 16L:8D on 2 August (i.e. observed 22 days after being placed at 16°C).

On 2 September, one nymph hatched from eggs kept at 0°C and one nymph from eggs kept at -5°C, all of which were placed at 16°C 16L:8D on 24 August (i.e. nymphs observed 9 days after being placed at 16°C).

Discussion

Conditions required for sexual morph induction and egg production
In this study, induction of the sexual morph and egg laying were consistently induced in the laboratory using conditions which simulated the average field temperature and photoperiod for September (12°C 13L:11D). This condition corresponded well with field observations where sexual morphs of *N. ribisnigri* were identified on blackcurrants in early October. The observed time interval of approximately 4-5 weeks between induction of the sexual morph on the secondary host and presence of the sexual morph on the winter host is likely to be due to the time needed for development and migration. This estimation was made using a date of sexual morph induction of 10 September (13L:11D photoperiod observed on this

date in 2009) and 14 October as the date they were present on the winter host (as observed in Experiment 4.1).

Table 4.7 summarises the outcomes from Experiment 4.2 and 4.3. Experiment 4.2 determined which morphs were produced when subjecting the embryos of an adult to pre-natal conditioning, and then following their birth, post-natal conditioning, at a range of rearing regimes (outcome from only one generation was observed). Where these rearing regimes resulted in the production of sexual morphs, Experiment 4.3 then maintained *N. ribisnigri* on lettuce plants paired with a blackcurrant cutting or plant to confirm whether eggs were produced over a longer period of time (outcome from several generations observed).

Table 4.7 A summary of the outcomes of Experiments 4.2 and 4.3 at each rearing regime. The natural conditions that each rearing regime is intended to represent are also described.

Rearing regime	**Natural conditions**	**Exp. 4.2- which morphs were produced**	**Exp. 4.3- whether eggs were produced**
15°C 14L:10D	Mid/late august	Males + apterae + alates	No
15°C 13L:11D	Late august to early September	Males + apterae + alates	No
12°C 16L:8D	Not a natural condition	Apterae + alates	Not included
12°C 14L:10D	Not a natural condition	Gynoparae + males + apterae + alates	Un-determined
12°C 13L:11D	September	Apterae + gynoparae	Yes
15°C 13L:11D transferred to 12°C 13L:11D	Late august and September	Not included	No - 66 days at 15°C Yes - 31 days at 12°C
15°C 13L:11D transferred to 12°C 13L:11D	Late august and September	Not included	Yes - 56 days at 15°C Yes - 13 days at 12°C

When reviewing the range of conditions which induced males and gynoparae in Experiment 4.2, males were induced prior to the gynoparae at 15°C 14L:10D and 15°C 13L:11D. They were produced at the same time as the gynoparae at 12°C 14L:10D. Gynoparae were produced alone at 12°C 13L:11D. However, when these outcomes are combined with the additional information provided by Experiment 4.3, various additional observations and contradictions occur:

1) Firstly, egg production was not confirmed at 12°C 14L:10D and it is therefore difficult to determine whether the three gynoparae recorded during the first two days of reproduction under these conditions (Table 4.2.3d) were not just parthenogenetic aphids which had failed to reproduce. While the induction of gynoparae is feasible at a 10 hr scotophase, as observed for *M. persicae* (Matsuka and Mittler, 1979), in this study it cannot be confirmed whether males and gynoparae were produced together under these conditions.

2) Secondly, it is uncertain whether eggs were produced at 15°C 13L:11D as two contradictory results were obtained in Experiment 4.3 (discussed further later). If eggs were produced it would indicate that gynoparae are produced under these conditions but that it took more than one generation for them to occur.

3) Finally, at 12°C 13L:11D eggs were obtained consistently in Experiment 4.3, which indicates that males were produced under these conditions, although this was not observed in Experiment 4.2. This suggests males occur in subsequent generations.

Using this information, Table 4.8 summarises the observations that can be made with confidence about the production of males and gynoparae under each set of conditions using the outcomes from both Experiments 4.2 and 4.3.

Table 4.8 The rearing regimes under which induction of males and gynoparae can be confirmed from this study.

Rearing regime	Males produced	Gynoparae produced
15°C 14L:10D	Yes	No
15°C 13L:11D	Yes	Unknown
12°C 16L:8D	No	No
12°C 14L:10D	Yes	Unknown
12°C 13L:11D	Yes	Yes

This study therefore supports the hypothesis that under natural field conditions, males are produced first in response to conditions which represent mid/late August and early September (15°C 14L:10D and 15°C 13L:11D respectively). It is likely that males continue to be induced at shorter scotophases/CNL than 10 h. This has been observed for males of *M. persicae* and *H. lactucae* which are induced at scotophases of 9 hr 26 min and 8 hr respectively (Matsuka and Mittler, 1979; Harrington, 1984). It is thought that the process of producing males first ensures that they are abundant on the winter host, so they are available to mate and fertilise the eggs of the oviparae after the gynoparae arrive (Matsuka and Mittler, 1979).

In September, the gynoparae are then induced in response to a longer CNL and lower temperatures (12°C 13L:11D). Similarly, studies on other species have shown that induction of gynoparae and oviparae requires a longer CNL than for males, which for *M. persicae* is 30 minutes longer at 18-19°C (Matsuka and Mittler, 1979) and >1 hour longer for *A. pisum* at 20°C (Lamb and Pointing, 1972). In this study, eggs were produced at 12°C 13L:11D and therefore males are produced together with the gynoparae at the same CNL. Similarly, this has been observed for other species of holocyclic aphid, where gynoparae and males of *A. fabae* and *R. padi* were induced at a similar CNL (Vaz Nunes and Hardie, 2000b). The present study suggests that gynoparae are produced in the first generation and males are produced in subsequent generations, as while eggs were produced in Experiment 4.3 (which allowed the

outcome for more than one generation to be observed), only males were observed in the first generation (Experiment 4.2). In other aphid species, males are usually produced after females, but during the same reproductive sequence (Dixon and Dewar, 1974; Lamb and Pointing, 1975; MacKay, 1987). Lamb and Pointing (1975) did show that sometimes the odd female does not produce males even under suitable conditions for their production, but in the present study none of the females produced males. No other investigations of sexual morph induction suggest differences in sexual morph production between generations and Mackay (1987) reported that by the G_2 generation (as used in this study) the effects of photoperiod on sexual morph production of *A. pisum* were fully developed and no changes occurred in successive generations. Therefore, this anomaly could be attributed to the lack of replication in Experiment 4.2 where by chance no gynoparae were produced. Further replications would be required to confirm this.

This study supports the findings of earlier studies in showing that photoperiod, more specifically the scotophase, as determined by Lees (1973), is the dominant factor controlling the induction of sexual morphs, since a low temperature and short scotophase regime (12°C 16L:8D) did not induce any sexual morphs. Vaz Nunes and Hardie (2000a) also found that male *A. fabae* were not produced at 12°C when the photoperiod was longer than 15 hours. Kenten (1955) also observed that no males or oviparae were produced by *A. pisum* kept at 11-13°C 16L:8D. However, the conditions which represent short day effects appear to be very different between species, with oviparae of *M. viciae* being produced at 12°C 16L:8D (Lees, 1959; Vaz Nunes and Hardie, 2000a). Other studies on *M. viciae* have recorded the production of 100% parthenogenetic aphids at 12°C 16L:8D which suggest that different clones of a species may express different responses (Hardie, 1990).

The use of both temperature and photoperiodic cues to detect changes in seasons, allows aphids to time their switch to a sexual life-cycle carefully, so the advantages of asexual reproduction are not lost prematurely and enough time remains for overwintering eggs to be deposited (Ward, *et al.*, 1984). However, it is possible that global climate change could lead to asynchrony between the sexual morph inducing cues. For example, at higher latitudes insects usually have a shorter CNL as winter occurs earlier, but as temperatures continue to increase as a result of climate change,

the CNL may no longer be an accurate indicator of seasonal changes in temperature (Bale and Hayward, 2010). Depending on the species, and the influence of higher temperatures on the CNL cue, this could result in aphids being unable to produce sexual morphs to enter the diapause process, or alternatively entering the diapause process too early.

Research into the effect of temperature on the CNL has shown that higher temperatures increase the CNL. This would postpone the induction of sexual morphs so that asexual reproduction continues for longer, potentially contributing to a larger overwintering population (Lamb and Pointing, 1972). Lees (1963) observed that higher temperatures (e.g. 25°C) could suppress the induction of sexual morphs. The effects of high temperatures have not been evaluated for *N. ribisnigri* as the higher temperature (15°C) used in this study was not unusually high compared with temperatures that would be experienced in the field during the period of induction of sexual morphs.

Further work could readily resolve the contradictions observed in determination of the requirements for sexual morph induction in this study. Precise CNLs could also be determined for each sexual morph by using treatments where the test scotophase is increased by short time intervals until sexual morphs are no longer produced. This could also be expanded to include other clones of *N. ribisnigri*, as there is evidence that the CNL can vary with the geographic origin of a clone, particularly related to latitude, so that clones from the north have shorter CNLs (Tauber and Tauber, 1972; Vaz Nunes and Hardie, 2000b). This would provide more specific information on the conditions required for sexual morph induction in *N. ribisnigri*.

Pairing infested lettuce plants and currant cuttings/plants at 12°C 13L:11D provided an effective method for obtaining eggs of *N. ribisnigri* and future studies can use this method to obtain eggs in approximately 49 days. During Experiment 4.3, when a regime of 15°C 13L:11D was evaluated, several eggs were observed after 56 days exposure, before the temperature was lowered to 12°C, which suggests that both males and females were produced under this rearing regime. However, no eggs were produced on another occasion using the same regime and, furthermore, when aphids were exposed to 15°C 13L:11D continuously, no eggs were produced after 63 days.

The results of Experiment 4.2 also suggest that only males were produced under this rearing regime. Together, the evidence suggests that it is unlikely that eggs were produced at 15°C 13L:11D. It is possible that the eggs which were observed were present on the blackcurrant plant when it was collected from the field and were not observed, and therefore removed, during its preparation for the experiment. Further experiments using this rearing regime would be required to clarify the short day effect of 15°C 13L:11D.

Once eggs had been produced, 16°C 16L:8D was a suitable hatching condition. During the egg hatch experiments, however, survival and viability were low. Relatively high humidity is vital for successful egg hatch, by preventing desiccation (Hand, 1983). Unfortunately, attempts to maintain a high humidity during this study were unsuccessful, with currant cuttings transferred to 16°C 16L:8D experiencing a mean humidity of 73.42% , where desiccation still occurred. A high humidity was maintained when eggs were conditioned at chilling temperatures (Experiment 4.6) and stored in sealed zipper plastic bags, using the method deployed by Lushai *et al.* (1996). Using this method, a mean humidity of 89.9% was maintained and the eggs appeared viable on transfer to 16°C 16L:8D.

The majority of previous studies on aphid eggs have maintained high humidity through the use of closed systems such as Petri dishes, the consistent addition of water and the use of controlled environments, which have all provided high rates of hatching (Newton and Dixon, 1987; Via, 1992; Wang and Furuta, 2002; Graf*, et al.*, 2006). Fungal infection was commonly observed in the present study, suggesting that in future, a procedure for egg sterilisation should be implemented. This has been very effective in reducing fungal infection and increasing the proportion of eggs hatching successfully (Wipperfűrth and Mittler, 1986; Via, 1992). However, regardless of these improvements in technique, eggs can still fail to hatch, even if they have developed fully and conditions are favourable, as observed for *A. fabae* (Way and Banks, 1964).

Egg monitoring and diapause termination

In the field, egg deposition by *N. ribisnigri* commenced during late October. This was followed by egg hatch by 17 and 24 February during 2011 and 2012

respectively. When eggs were sampled from the field on 1 February 2011 and 20 January 2012 and transferred to suitable hatching conditions throughout the winter, they hatched earlier, by 9 and 1 February during 2011 and 2012 respectively. This is similar for many other species where in the field, hatching of *R. insertum and Cinara pilicornis* eggs were delayed by temperatures too low for development (known as quiescence) even though diapause had terminated (McLeod and Beck, 1963; James and Luff, 1982). Therefore, if climate change results in spring conditions becoming more favourable, *N. ribisnigri* may commence hatching earlier in the field than currently observed. Earlier hatching will provide the aphids with more time to develop and reproduce, potentially leading to higher summer populations.

When comparing field-produced and laboratory-produced eggs used during 2011/2012 in Experiment 4.5, the laboratory-produced eggs hatched earlier. With the only difference between the eggs being the time of oviposition, this suggests that the timing of egg deposition influences the length of the diapause and therefore the timing of diapause termination and subsequent hatching (Leather, *et al.*, 1993). This might also explain the differences in the timing of egg hatch in the field during 2011 and 2012. This needs to be considered in future studies.

When sampling eggs from the field and transferring them to 16°C 16L:8D, the first field-produced eggs hatched in 2010/2011 after a period of 96 days in the field and 7-8 days at 16°C 16L:8D following sampling on 1 February 2011 (Table 4.9- 28 October was used as the oviposition date as this is the date eggs were first observed in the field during 2011). Following the first hatch, eggs transferred on 17 February, 25 February and 4 March 2011 took 4, 3 and 3 days respectively to hatch at 16°C 16L:8D. During 2011/2012, the first egg hatch was recorded after 84 days in the field and 11-12 days at 16°C 16L:8D following sampling on 20 January 2012 (Table 4.9). Following the first egg hatch, eggs transferred on 30 January, 13 February and 24 February 2012 hatched after 4, 4 and 3 days at 16°C 16L:8D.

Table 4.9 Summary of the time (days) eggs were kept in treatment conditions (0, -5 °C or the field), the date they were transferred to 16°C 16L:8D and the duration of incubation before the first egg hatched (Experiment 4.5 and 4.6).

Treatment and date of transfer to 16°C 16L:8D	Time (days) eggs kept in treatment conditions	Time (days) eggs kept at 16°C 16L:8D*	Exp.
Field-produced eggs 2010/2011	96	7-8	4.5
Field-produced eggs 2011/2012	84	11-12	4.5
Laboratory-produced eggs 2011/2012	70	14-17	4.5
0°C (transferred on 2 August 2012)	55	17-22	4.6
0°C (transferred on 24 August 2012)	77	6-9	4.6
-5°C (transferred on 24 August 2012)	77	6-9	4.6

*Estimates of incubation time at 16°C 16L:8D took into account the time period between when egg hatch was recorded and the prior sampling date, as egg hatch could have occurred between these two dates where no monitoring took place.

It is clear, for the field-produced eggs, that the longer the eggs remained in the field, a shorter period was required subsequently at 16°C 16L:8D before they hatched, with the shortest incubation time being three days. Due to temperature variation in the field, fluctuating above and below development thresholds, the exact point at which diapause terminated cannot be determined, as while the eggs hatched following transfer to 16°C 16L:8D, it cannot be assumed that diapause had terminated prior to their transfer. Therefore, it can only be confidently concluded that

diapause terminated within 104 days (time until egg hatch) during 2010/2011 and within 96 days during 2011/2012.

Although the point at which diapause terminated cannot be determined from this study, an estimate of the number of DD required for post-diapause development can still be made using the period of time between the sampling date and date of egg hatch. Day-degrees accumulated between the sampling date and date of egg hatch for field-produced eggs in 2010/2011 and 2011/2012 and also laboratory-produced eggs during 2011/2012 are shown in Figure 4.10. The estimates were calculated using the LDT at 4.7°C, as determined for adult *N. ribisnigri* in Chapter 3, and the DD measured from the 1 December during 2010/2011 and 2011/2012 (using minimum and maximum temperatures collected from the University of Warwick, Wellesbourne meteorological station). Day-degrees were calculated from the sampling date as eggs might have terminated diapause in the field and accumulated day-degrees prior to incubation. Therefore, this estimate in some cases may be an overestimation.

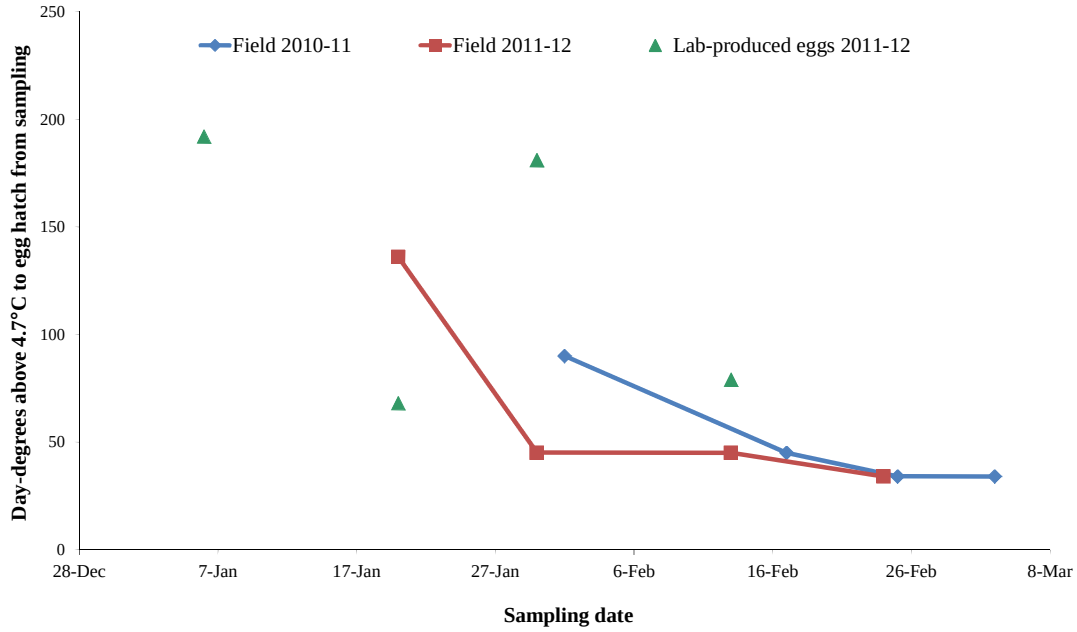


Figure 4.10 Day-degrees accumulated between the sampling date and date of egg hatch for field-produced eggs in 2010/2011 and 2011/2012 and laboratory-produced eggs during 2011/2012.

When looking at the data for the field-produced eggs during 2010/2011 and 2011/2012, Figure 4.10 shows that the time required until egg hatch at 16°C 16L:8D is becoming constant at approximately 50DD. Therefore, it can be estimated that post-diapause development takes just under 50 DD using a LDT of 4.7°C at 16°C, and that diapause terminates sometime in late January – early February.

Unlike the field-produced eggs, the data for the laboratory-produced eggs were more variable. The first egg hatched after 70 days in the field and 14-17 days at 16°C 16L:8D following sampling on 6 January 2012 (Table 4.9). Following this, eggs transferred on 20 January, 30 January and 13 February took 6, 16 and 7 days to hatch at 16°C. Therefore, this does not indicate that the longer the eggs were in the field before they were transferred to 16°C 16L:8D, the shorter the amount of time they then required before they hatched. The cause of this anomaly cannot be determined as the data in this study is based on a very small sample and low number of these eggs hatched. Studies have shown that intra-population variation in the timing of egg hatch does exist, with early and late hatching aphid phenotypes being observed for the sycamore aphid for example (Dixon, 1976; Tauber*, et al.*, 1986). While this could explain the variation observed in these results, the lack of data meant that intra-population variation could not be observed.

To determine the diapause termination and egg hatch requirements more precisely for *N. ribisnigri*, eggs need to be kept at a constant temperature below the threshold for development. Batches of eggs should then be removed at intervals to higher temperatures that support post-diapause development so that the time until egg hatch can be measured. Once the time to egg hatch becomes constant (i.e. all eggs take 5 days to hatch) it can be confirmed that diapause has terminated and therefore the time to egg hatch represents the egg development time at that temperature. Wang and Furuta (2002) define egg development time (days) as the number of days to hatching following transfer to warmer temperatures.

In this study, a similar method was used where eggs were chilled at 0 and -5°C from 8 June 2012 (assumed to be below the egg developmental threshold) and transferred to 16°C 16L:8D at weekly intervals (Experiment 4.6). Table 4.9 summarises the time kept at the chilling condition and at 16°C 16L:8D. For eggs transferred from 0°C to 16°C 16L:8D on 2 August 2012 diapause took no longer than 77 days at 0°C to

terminate (8 June 2012 used as the start date of diapause). For eggs transferred from 0 and -5°C to 16°C 16L:8D on 24 August 2012 diapause took no longer than 86 days to terminate.

Unfortunately, egg hatch was only observed from these two sampling dates as there were insufficient eggs available to continue sampling. Therefore, a constant emergence time at 16°C 16L:8D was not observed. Thus it could not be confirmed that diapause had terminated prior to transfer to the incubator and as a result, egg development time could not be determined. If these data had been obtained, the development times could have been converted into day-degrees to provide a prediction of *N. ribisnigri* emergence in the spring using the LDT estimate of 4.7°C. Future work should consider repeating this experiment, and also adapting it to determine the LDT specifically for egg development, as using the LDT for the adult stage may not be appropriate for the egg stage. This was observed by Graf *et al.* (2006) who found a 0.5ºC difference between the LDT of nymphs and eggs of *Dysaphis plantaginea*.

The LDT for egg development could be determined by introducing a range of incubating temperatures, in addition to 16°C, to determine the effect of temperature on egg development time. The development times could then be converted to a development rate and plotted against temperature, so that the LDT could be extrapolated using the x-intercept method as used in Chapter 3, and as demonstrated by Wang and Furata (2002) for the eggs of *Periphyllus californiensis* and Graf *et al.* (2006) for the eggs of *D. plantaginea.*

Studies which have compared egg hatch patterns between years have found that temperature has a significant influence for many insects (Dixon, 1976; Wang and Furuta, 2002). It is hypothesised for aphids that the effect of temperature is related to their chilling requirements, as discussed in the Introduction, which subsequently affects the timing of diapause termination. This study shows that diapause development in *N. ribisnigri* is influenced by temperature, with differences between field temperatures and experimental chilling conditions being observed. However, studies on *R. insertum* and *P. californiensis* have found that eggs will not hatch until after mid-January or 35-41 days respectively, regardless of whether the eggs were kept in the field or chilled in the laboratory, suggesting that temperature may affect

diapause differently in some species (James and Luff, 1982; Wang and Furuta, 2002).

While Experiment 4.6 only provided a small amount of data, the study did show that diapause development progressed more quickly for eggs chilled at 0°C compared to those chilled at -5ºC and sampled from the field (Table 4.9). This could suggest that the rate of diapause development changes with temperature.

Temperature has been found to regulate diapause development for various aphid species where for example, eggs of *A. fabae* required 48-52 days below 8ºC to hatch (Newton and Dixon, 1987) and eggs of *S. avenae* failed to hatch when maintained above 10ºC (Hand, 1983). Studies have also suggested that temperature regulates the rate of insect diapause development, with low temperatures in particular, resulting in the most rapid completion of diapause. For example, the western cherry fruit fly (*Rhagoletis indifferens*) has an optimum temperature of 3ºC for diapause development (Vankirk and AliNiazee, 1982), and the diapause development of *Delia radicum* pupae progressed most rapidly when temperatures were lower within a 0-10ºC range (Collier and Finch, 1983).

As *N. ribisnigri* eggs under natural field conditions would not be exposed to a single chilling temperature of 0ºC during the winter for 55 days, it suggests that a range of temperatures must be facilitating diapause development as observed for the examples above. While for some species, subzero temperatures do not facilitate diapause development, in this study a -5°C condition did, although it was not the optimum temperature for diapause development (James and Luff, 1982; Hand, 1983; Lushai, *et al.*, 1996; Wang and Furuta, 2002).

A low temperature threshold mechanism such as this would explain why diapause development of *N. ribisnigri* eggs progressed faster when eggs were kept at 0°C compared with -5°C or under field conditions. To confirm that lower temperatures regulate and determine the rate of diapause development in *N. ribisnigri,* the chilling experiments would need to be repeated including a range of low and high temperatures. It is also important to determine whether the optimum and effective temperature ranges rise as diapause development proceeds, as this has been suggested by Tauber and Tauber (1976) to be a diapause terminating mechanism.

Data from Experiment 4.6 also suggested that chilling reduced egg development time. Eggs chilled at 0°C required 22 days to hatch when transferred to 16°C 16L:8D while eggs chilled for 77 days only required 9 days to hatch. While this conclusion is drawn from two sampling dates only, similar observations have been made for other species (Wipperfűrth and Mittler, 1986; Komazaki, 1998; Wang and Furuta, 2002; Chuche and Thiéry, 2009). In addition, more detailed studies might record improvements in hatching success in response to longer chilling periods, as observed in other studies on aphids (Hand, 1983; Lushai, *et al.*, 1996; Wang and Furuta, 2002).

Unfortunately, this study cannot confirm that chilling temperatures acted to terminate diapause as it is unknown whether *N. ribisnigri* eggs can hatch when maintained at higher temperatures. While future work could determine this, observations of hatching at higher temperatures may be difficult to interpret, as in some insect populations, a small number (3%) of non-diapausing genotypes have been observed (Vankirk and AliNiazee, 1982; Thomas, *et al.*, 2012;). The occurrence of non-diapausing genotypes could explain why some studies on aphid eggs have reported hatching at constant high temperatures such as 16ºC (Wipperfűrth and Mittler, 1986).

Following egg hatch in the field during 2012, *N. ribisnigri* did not establish successfully on blackcurrant plants, and it was clear that egg hatch was not synchronised with bud burst and that the aphids had hatched too early. The reason for this asynchrony could be due to climate change, leading to an increasing frequency of milder winters, which are resulting in delayed blackcurrant bud break. This is due to a lack of bud chilling, which is a requirement for bud burst (Jones, *et al.*, 2012). Jones *et al.* (2012) expressed these concerns particularly in relation to *Ribes nigrum* L. (blackcurrant).

Insects have been observed to implement ‘risk-spreading strategies’ where the existence of early and late hatching phenotypes helps them to contend with early and delayed bud opening, or temporarily unfavourable conditions (Hopper, 1999; Tauber, *et al.*, 1986). *Drepanosiphum platanoidis* (sycamore aphid) is an example of an aphid which adopts this strategy (Dixon, 1976). However, an extreme delay in bud hatch can reduce survival (Dixon, 1976). Fundatrices hatching before bud burst

have been reported to suffer high mortality as they feed on the bud scales where they are vulnerable to predators (particularly birds and other arthropods) and rain (Dunn and Wright, 1955; Dixon, 1976; Gange and Llewellyn, 1988).

The survival of fundatrices is also influenced by the age and quality of the leaves. Dixon (1976) observed that fundatrices of the sycamore aphid emerging and feeding on unfurling and young leaves had a superior weight and reproductive rate, compared to fundatrices feeding on older mature leaves of the sycamore tree. In the present study, fundatrices placed on unfurling leaves of blackcurrant cuttings (to determine development time) died within a few days, as their subsequent feeding resulted in leaf curling and discolouration resulting in a poor quality food source. Similar influences of plant age and quality have been shown for *R. padi* where they survived less than four days on mature and senescent leaves of bird cherry (Leather, 1980).

In a changing climate, the adaptability of a species' life-cycle can be important for its survival. Following the observations in this study that hatching of *N. ribisnigri* eggs can be asynchronous with bud development of its primary host, investigations were carried out to determine how adaptable its life-cycle could be. Originally, it was hypothesised that specialisation of the fundatrix to the primary host restricted the transfer of an aphid's lifecycle from holocyclic to autoecious (Dixon, 1998). However, various holocyclic aphid species have been reared successfully on their secondary host plant following hatching, confirming that they are not as restricted to the primary host plant as originally thought (Dixon and Kundu, 1994). Furthermore, research on *Cavariella aegopodii* has demonstrated that, under experimental conditions, it can complete its entire life-cycle on its secondary host, with both the sexual morphs and fundatrices accepting the secondary host (Kundu and Dixon, 1995).

In the laboratory, the fundatrices of *N. ribisnigri* developed and reproduced successfully on lettuce and continued to produce offspring, which also continued to develop and reproduce on lettuce. When these offspring were transferred to the primary host they no longer reproduced, and died within a couple of days, suggesting no feeding occurred. Apterous and alate parthenogenetic *N. ribisnigri* females also did not accept the primary host plant when transferred from lettuce. In the case of the

primary host, apterous and alate parthenogenetic females of *C. aegopodii* survived on willow which highlights that there are differences in morph specialism between aphid species (Kundu and Dixon, 1995).

As it was not determined whether the males and gynoparae of *N. ribisnigri* could mate and lay eggs on the secondary host plant, it cannot be confirmed whether the life-cycle could be completed on lettuce (or other secondary hosts) alone. However, if this was confirmed then this could provide *N. ribisnigri* with the opportunity to become autoecious and shift its entire lifecycle to the secondary host, which has been observed for other members of the *Nasonovia* genus which became disconnected from *Ribes* species (Moran, 1992). As some aphid species which are not constrained to being holocyclic continue to remain so, host alternation must result in benefits, such as population increases, which outweigh the costs incurred when migrating between the hosts (Dixon and Kundu, 1994).

This study has made primary investigations into the overwintering phase of the life-cycle of *N. ribisnigri*, providing information on the timing of key events, as summarised in Figure 4.11, and has also attempted to provide explanations for the mechanisms controlling them. Techniques have been developed to induce sexual morphs, obtain eggs and hatch them successfully in the laboratory. These will be valuable tools for future studies, particularly the development of a forecast for egg hatch. Future work should aim to determine the requirements for diapause termination and egg hatch and also determine the LDT for eggs. On gaining this information, a day-degree forecast for spring emergence can be developed.

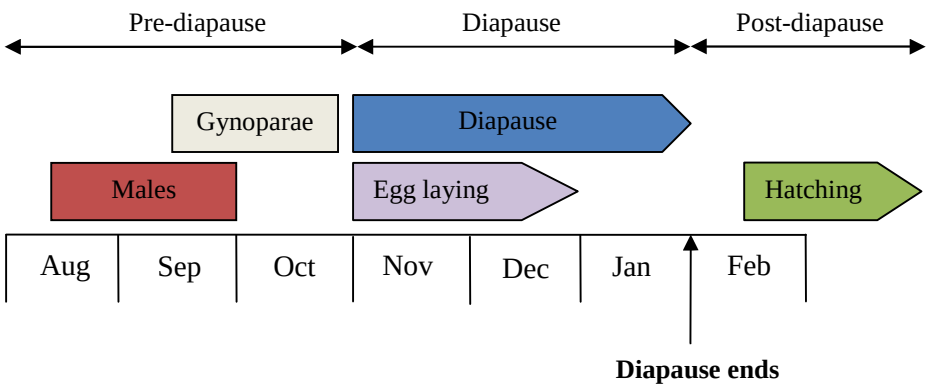


Figure 4.11 *Nasonovia ribisnigri* overwintering life-cycle.

Conclusion

- Diapause terminates in the field during mid-late January but temperature below the LDT prolonged post-diapause development and hatching until early February.
- 12°C 13L:11D can induce males and gynoparae of *N. ribisnigri* which produce eggs after approximately 49 days.
- It was estimated that post-diapause development takes just under 50DD using a LDT of 4.7°C at 16°C.
- It is hypothesised that diapause development is regulated by low temperatures. Diapause development progressed faster at 0ºC.
- A prolonged chilling period at 0°C reduced egg development time.
- *N. ribisnigri* fundatrices can survive and reproduce on lettuce but their offspring can no longer colonise *R. nigrum.* Parthenogentic summer alates and apterous *N. ribisnigri* cannot colonise blackcurrant.

Chapter 5: Alternative Host Plants

Introduction

The host plants aphids utilise complement their complex reproductive strategies (Moran, 1988). For holocyclic aphids, which carry out sexual and asexual reproduction, the primary woody winter host is vital for supporting overwintering eggs resulting from sexual reproduction, and the secondary herbaceous summer hosts provide a better nutritional source to support asexual colonization (Moran, 1992).

Host plant selection by aphids is a complex process involving; host plant location, plant contact and assessment, epidermal probing, stylet pathway activity, sieve element entry and salivation, concluding with phloem acceptance and ingestion (Powell, *et al.*, 2006). At the beginning of this process, aphids are known to locate plants via visual cues (Kennedy, *et al.*, 1961), but growing evidence suggests that olfactory cues also play a role in host plant location, where aphids have been attracted to and altered their flight paths in response to host plant odours/volatiles (Pickett, *et al.*, 1992; Webster, *et al.*, 2008). Following host plant location, aphids alight on the leaf and recognise that the host plant is appropriate via responses to physical and chemical properties of the leaf surface (Dixon, 1998; Powell, *et al.*, 1999) and/or following epidermal penetration (Powell and Hardie, 2000; Vargas, *et al.*, 2005). At this point some aphids accept the host plant, and evidence for this pre-phloem acceptance is strongly supported by the induction of reproduction before the sieve elements are reached, which has been recorded for *R. padi* and *A. fabae* (Nam and Hardie, 2012; Tosh, *et al.*, 2002).

Collectively aphids utilise a wide range of host plants, but the majority are host-specific with a genetically determined preference for one, or several closely related, species (Dixon, 1998). This limited range of host plants makes locating food sources challenging and this is exacerbated by the difficulties experienced by aphids in achieving directed flight in strong winds and the high risks of desiccation during off-plant activity (Nam and Hardie, 2012).

When utilising plants, aphids can be monoecious, where they remain on one host plant species, or heteroecious, remaining on several host species (Wöhrmann and Tomiuk, 1988). The species studied most are the heteroecious host alternating species, as their herbaceous secondary host is often an important agricultural crop, which is the case for *N. ribisnigri* (Williams and Dixon, 2007).

Nasonovia ribisnigri is a host-alternating holocyclic aphid and its main secondary host plants are from the Asteraceae, particularly *Lactuca* species, which includes economically important commercial lettuce cultivars. In addition, *N. ribisnigri* has been recorded on other plant families including Brassicaceae, Scrophulariaceae and Solanaceae (Blackman and Eastop, 2006b).

Increasing host plant range is beneficial for aphids as they have the opportunity to survive when their preferred host plants are limited. This is particularly important for anholocyclic aphids which reproduce asexually all year round, surviving as live stages in the winter (Williams and Dixon, 2007). This is advantageous when primary hosts are absent, or the original secondary hosts are sparse (Phillips, *et al.*, 1999). *Myzus persicae*, for example, is holocyclic and undertakes sexual reproduction to lay overwintering eggs on peach trees, *Prunus persica*. However, in regions where there is an absence of peach trees, Blackwell (1974) identified selection for anholocylic clones, which overwinter on alternative herbaceous plants, weeds and winter crops (Blackman, 1974; Margaritopoulos, *et al.*, 2002). In addition, regions which exhibit increasingly milder winters may also be selecting against sexual reproduction, indicating that climatic variation between geographical locations plays an important role in determining the overwintering life-cycle of aphids (Blackman, 1974; Powell and Bale, 2004).

The advantage to aphids of the existence of both sexual and parthenogenetic reproduction has been discussed extensively. It is widely accepted that there is a two-fold cost of sex, where due to the production of males, only half of a sexually-reproducing population can produce offspring, compared with an asexual population where all individuals are capable (Smith, 1971). For aphids, the cost of sex far exceeds a two-fold cost as asexual aphids can pass through several generations in the time required for one generation to be produced during a sexual phase (Newton and Dixon, 1988; Simon, *et al.*, 2002). Apart from the advantages of maintaining genetic

variation (Dixon, 1977), selection for sexual reproduction is maintained for the production of cold resistant eggs, which ensures their winter survival and presence in spring (Vorburger, 2004). For anholocyclic clones, overwintering survival is not guaranteed and the number of migrants is often low in spring due to high mortality throughout the winter (Powell and Bale, 2008).

Interestingly, to avoid this risk of mortality in varying climates, some anholocyclic lineages of *S. avenae*, in addition to producing parthenogenetic females, have retained their ability to produce all of the sexual morphs, earning them the title of 'intermediate' aphids (Simon, *et al.*, 2002). In addition, some clones may produce only males and parthenogenetic offspring and are termed androcyclic (Simon, *et al.*, 1999). These varying commitments to sexual reproduction are advantageous to the species, as their subsequent mating allows gene flow to occur, and protects their genes in less favourable environments through the production of an overwintering egg (Rispe and Pierre, 1998). Therefore, the existence of these different lineages indicates that the change in the life-cycle is under genetic control, regulated by the environment (Wöhrmann and Tomiuk, 1988).

Over the past few years there have been an increasing number of reports that in some temperate regions with mild winters, including the south of England, that *N. ribisnigri* has survived through the winter as active stages on alternative hosts to *Ribes* species (RIS, No date-a). This is a cause for concern, especially during very mild winters, since development of large populations can occur earlier in the spring as reproduction continues throughout the winter (Wales, *et al.*, 2008).

The aim of this study was to investigate and confirm potential alternative host plants, including relatives of lettuce and other wild species, which are suitable hosts for UK populations of *N. ribisnigri*. A selection of these hosts was then assessed to determine if they could overwinter and support the development of *N. ribisnigri.*

Methods

5.1 Host plant screening

Plants to be screened were selected firstly by focusing on broad leaved weeds which are considered very important in field vegetable crops and could potentially provide refuges for *N. ribisnigri* (HDC, 2009). Other plants were chosen by using published information on aphid-host associations and confirming that these plants inhabit areas close to agricultural environments (Blackman and Eastop, 2006a; Blackman and Eastop, 2006b). All the plants chosen were eudicots covering five orders and seven families. Table 5.1.1 lists the plants selected. Seeds were acquired from Nicky's Nursery Ltd (Kent), Herbiseed (Twyford) and Chiltern Seeds Ltd (Wallingford). More plant species were identified, but seeds could not be sourced.

Preliminary work was carried out to determine the best method to germinate each host plant and the plant age most suitable for the screening experiments. Seeds were stratified in a fridge prior to use. Various methods were then used to stimulate germination; 1) sowing in soil (3 seeds per pot), 2) sowing in soil and covering the seeds, 3) germinating in vermiculite and transplanting into soil and 4) treatment of the seeds with gibberellin (GA) to stimulate germination prior to sowing in soil (seeds were immersed in 2.5% bleach followed by rinsing in sterile water, each seed was then treated with 7µl of GA). The most suitable plant age (days when grown at 18°C 16L:8D) was selected by visually determining when the plant could support an aphid colony based on previous experience.

Table 5.1.1 Selected plants including binomen name, vernacular name and plant family.

Binomen name	Vernacular name	Plant family
Agrostemma githago	Corn cockle	Caryophyllaceae
Capsella bursa-pastoris	Shepherd's Purse	Brassicaceae
Chenopodium album	Fat Hen	Amaranthaceae
Chichorium intybus	Chicory	Asteraceae
Cirsium arvense	Creeping Thistle	Asteraceae
Crepis capillaris	Smooth Hawksbeard	Asteraceae
Euphrasia nemorosa	Eyebright	Scrophulariaceae
Lapsana communis	Nipplewort	Asteraceae
Galium aparine	Clevers	Rubiaceae
Hieracium aurantiacum	Orange Hawkweed	Asteraceae
Hieracium pilosella	Mouse-ear Hawkweed	Asteraceae
Persicaria maculosa	Redshank	Polygonaceae
Polygonum aviculare	Common Knotgrass	Polygonaceae
Senecio vulgaris	Common Groundsel	Asteraceae
Sinapsis arvensis	Wild Charlock Mustard	Brassicaceae
Sonchus arvensis	Field Sowthistle	Asteraceae
Sonchus asper	Prickly Sowthistle	Asteraceae
Sonchus oleraceus	Smooth Sowthistle	Asteraceae
Stellaria media	Common Chickweed	Caryophyllaceae
Veronica arvensis	Wall Speedwell	Scrophulariaceae
Veronica hederifolia	Ivy-leaved Speedwell	Scrophulariaceae
Veronica spicata	Spiked Speedwell	Scrophulariaceae
Veronica officinalis	Common Speedwell	Scrophulariaceae
Veronica persica	Common Field Speedwell	Scrophulariaceae

Sixteen host plants germinated successfully and were used in the screens. *Veronica persica, V. hederifolia, P. aviculare, E. nemorosa, C. album* and *G. aparine* failed to germinate despite using all germination methods and were not included in the screens. *Cirsium arvense* and *P. maculosa* had very poor germination rates and did not produce enough plants to be used in the screen, but a preliminary screen was carried out for these species. The effective germination methods and appropriate plant ages for testing can be seen in Table 5.1.2.

Table 5.1.2 Germination method and appropriate plant age from sowing

Host plant	Optimum germination method	Plant Age
A. githago	Germinate in vermiculite 18°C 16L:8D	83 days
C. bursa-pastoris	Sow in soil and cover at 18°C 16L:8D	59 days
C. capillaris	Sow in soil at 18°C 16L:8D	27 days
C. intybus	Sow in soil and cover at 18°C 16L:8D	27 days
C. arvense	Germinate in vermiculite 18°C 16L:8D (poor germination)	27 days
H. aurantiacum	Sow in soil and cover at 18°C 16L:8D	30 days
H. pilosella	Sow in soil at 18°C 16L:8D	27 days
L. communis	Germinate in vermiculite 18°C 16L:8D	30 days
P. maculosa	Germinate in vermiculite 18°C 16L:8D (poor germination)	27 days
S. asper	Sow in soil at 18°C 16L:8D	27 days
S. media	Sow in soil and cover at 18°C 16L:8D	59 days
S. oleraceus	Sow in soil and cover at 18°C 16L:8D	40 days
S. vulgaris	Sow in soil at 18°C 16L:8D	70 days
Sinapsis arvensis	Sow in soil and cover at 18°C 16L:8D	59 days
Sonchus arvensis	Sow in soil and cover at 18°C 16L:8D	30 days
V. arvensis	Sow in soil at 18°C 16L:8D	45 days
V. officinalis	Sow in soil and cover at 18°C 16L:8D	30 days
V. Spicata	Sow in soil and cover at 18°C 16L:8D	30 days

Host plants were screened in three batches on 4 August 2011 (Batch 2), 6th October 2011 (Batch 3) and 14th November 2011 (Batch 1 – had to be repeated as *S. oleraceus* failed to germinate). Each batch consisted of six potential host plant species (including the control *C. intybus*) which were screened with both WT (4850a) and Rb *N. ribisnigri* (RbKent) biotypes, giving 12 treatments. Plants were arranged in a randomised complete block design with 12 treatments, 5 blocks and 60 plants in total. Plants were divided into batches to make data collection manageable and *C. intybus* was used as the control due to it being a relative of lettuce and a confirmed alternative host for *N. ribisnigri* (Blackman and Eastop, 2006a; RIS, No date-a) .

The plants were infested with new born nymphs of both WT and Rb *N. ribisnigri* which were obtained by inoculating 70 alates from each clone into separate cages containing five lettuce plants (cv. Saladin). These were left for 24 hours at 20°C 16L:8D to produce nymphs of similar ages.

Five plants of each species were then each inoculated with five new born WT *N. ribisnigri* nymphs and the remaining five were inoculated with Rb nymphs using a fine paint brush. The plants were then covered with micro-perforated polypropylene bags (200mm x500mm; Cryovac®) secured with an elastic band and kept at 20°C 16L:8D (IRU Room 3). The plants were left for three weeks, after which they were sampled destructively, and the numbers of *N. ribisnigri* were recorded, being separated into the number of alate aphids and the number of other aphids (apterous adults and nymphs).

Cirsium arvense and *P. maculosa* had very poor germination rates and only four of each of these species germinated. On 1st November 2011, a preliminary experiment was carried out with these plants. Two plants were each inoculated with five WT *N. ribisnigri* biotypes and the remaining two were each inoculated with five Rb biotype aphids. These were then left for three weeks as above.

5.2 Overwintering host plant experiment

Cichorium intybus, C. capillaris, V. arvensis and *L. communis* were planted outside on 29th and 30th November 2011 and inoculated with the WT *N. ribisnigri* biotype

(clone 4850a) to confirm whether the plants could survive the winter and whether *N. ribisnigri* could overwinter on them. These plant species were selected as they were suitable host plants for *N. ribisnigri* (as determined in Experiment 5.1), and provided a good representation of the morphological variation present in the family of Asteraceae and Scrophulariaceae (*V. officinalis* representing the family Plantaginaceae was not used as it had a poor germination rate).

Fifty two plants of *V. arvensis* and *L. communis,* and 76 of *C. intybus* and *C. capillaris* were grown from seed as described in Table 5.1.2. *Veronica arvensis* was sown on 12 October 2011, *L. communis* on 22 October 2011 and *C. intybus* and *C. capillaris* on 26 October 2011.

On 29 November 2011, 52 of each of these plants were transplanted in a split plot design into an unsheltered soil bed of an old Dutch Light at The University of Warwick, Wellesbourne campus (Figure 5.2.1a). Three days prior to transplanting, the plants were moved to IRU Room 5 at 10°C 16L:8D to acclimatise to a lower temperature and harden off. When used, *C. intybus* and *C. capillaris* were 33 days, *L. communis* 45 days and *V. arvensis* 38 days old. Plants were given a longer growing period than in Experiment 5.1 so they were more established and less vulnerable to winter conditions. Twenty six plants each of *C. intybus* and *C. capillaris* were also planted in two sheltered cages (2m x 3m) to determine the effects of shelter on aphid overwintering (Figure 5.2.1b).

WT *N. ribisnigri*, which had been kept outside in a cage for four days to acclimatise, were used. A total of 260 Eppendorf tubes were each filled with eight 4th instar nymphs and apterous adults, which were inoculated onto each of the host plants following transplanting.

Every week, four plants of each species were destructively sampled from the unsheltered sites and two of each species from the sheltered site, over a 13 week period beginning on 6 December 2011 (there was a two-week sampling interval between 20 December 2011 and 12 January 2012) and ending on 8 March 2012. Sampling was random, as directed by the design, and the numbers of *N. ribisnigri* were recorded, noting the numbers of alates, apterous adults and nymphs.

a)  **b)** 

Figure 5.2.1 a-b a) Unsheltered soil bed **b)** Sheltered cage.

Tiny Tag© loggers were used to record the temperature in both the sheltered and unsheltered sites to determine whether there were any differences in conditions, due to the presence of the cages, which could influence aphid development and survival. Readings were taken every 30 minutes from 30 November 2011 to 7 March 2012.

Results

5.1 Host plant screening

Analysis of the number of alates and the total number of *N. ribisnigri* (all stages) in batches one and two were performed separately using ANOVA, as the experiments were independent of each other. Due to the presence of zero value treatment means in the data set, two ANOVAs were carried out. Both used a LOG10 data transformation, but one of these added the value of one to the data set which ensured that treatments with zero values were included, while the LOG10 transformation alone considered the zero value treatments as missing data and created predicted values, which were not included in the analysis. An interpretation of the data was made using both treatment means and 5% LSD values. The two ANOVAs were carried out because including zeros does not meet the criteria for ANOVA, where the variances of grouped data should be the same. By comparing the two analyses with and without zeros, it can be determined whether including zero treatment means

results in significant overestimations of the differences between treatments. If no significant overestimations occur, the zero values can be analysed with the other treatments.

Batch one analysis

Batch one consisted of the host plants: *C. intybus, S. arvensis, H. pilosella, L. communis, V. officinalis* and *S. oleraceus.* The ANOVA, taking into account the zero values, showed that there was no effect of aphid biotype on the numbers of *N. ribisnigri* (all stages) per plant, but a statistically significant effect was seen for host plant ($F(5,44)= 589$, $p<0.001$), and the interaction between host plant and aphid biotype ($F(5,44)= 4.02$, $p=0.004$). When considering the number of alate *N. ribisnigri,* a statistically significant effect was seen only for host plant ($F(5,44)= 60.90$, $p<0.001$).

Figure 5.1.3 shows the transformed means and back-transformed means for the numbers of alates and all stages of WT and Rb *N. ribisnigri* per plant on each treatment after three weeks. Zero back-transformed values are not presented as they cannot be plotted on a LOG scale. Transformed values were used to interpret the analysis.

When analysing the number of *N. ribisnigri* (all stages) per plant, the control (WT biotype and *C. intybus*) was the best performing treatment, with the most *N. ribisnigri* (all stages) per plant, and was significantly different to all other treatment combinations screened, apart from the Rb biotype also on *C. intybus*.

WT *N. ribisnigri* survived on all host plants except *S. oleraeus* and *S. arvensis,* where only a small number of Rb *N. ribisnigri* were recorded on both, indicating they are unsuitable host plants for the WT biotype in particular. The analysis suggests that overall, WT and Rb biotypes performed similarly on each of the host plants screened; except that the Rb biotype had significantly more individuals (all stages) per plant than the WT biotype on *S. arvensis.*

When looking at the number of alate *N. ribisnigri* per plant, the Rb biotype on *C. intybus* had the highest number of alates, followed by the WT biotype (control). The control treatment was significantly different to all other treatment combinations except for its Rb counterpart and both aphid biotypes on *L. communis*. No

differences in alate numbers were observed between the Rb and WT aphid biotypes on each host plant, meaning they performed similarly. The least number of alates was found on *S. oleraceus*, with zero counts of Rb and WT alates, while a small number of Rb alates were found on *S. arvensis.* *Sonchus oleraceus* and *S. arvensis* were therefore unsuitable host plants, for the WT *N. ribisnigri* biotype in particular.

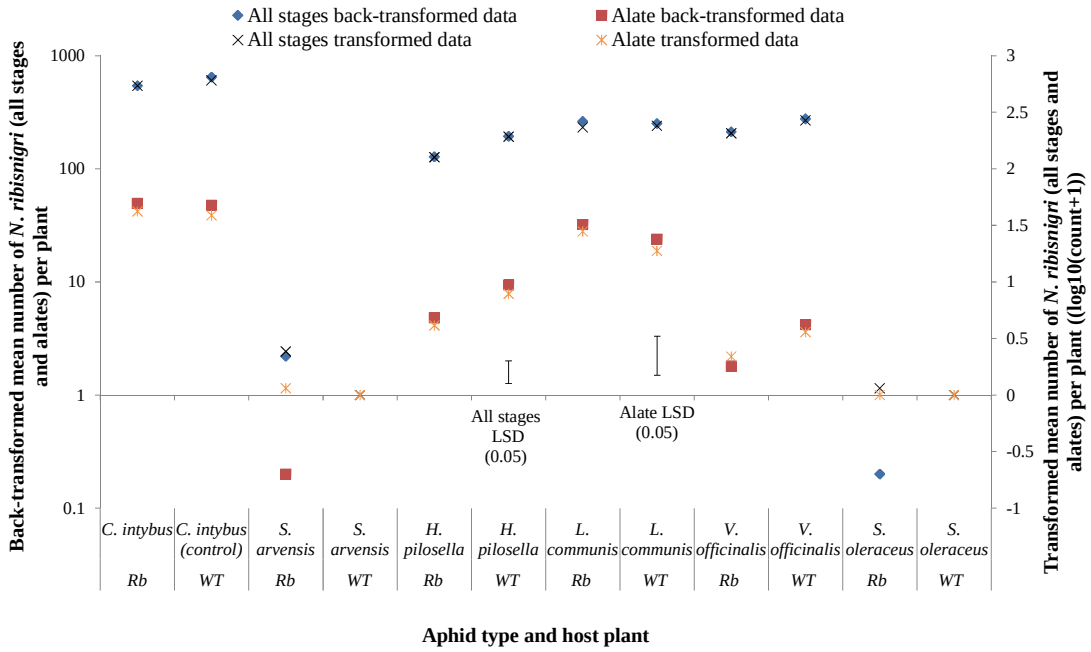


Figure 5.1.3 Transformed and back-transformed mean numbers of alate and all stages of WT and Rb *N. ribisnigri* present after three weeks in Batch one.

For the ANOVA which excluded the zero values and generated predicted values, a significant effect of aphid biotype ($F(1,30)= 7.48$, $p=0.010$) and host plant ($F(5,30)= 772.16$, $p<0.001$) were observed for the number of all stages of *N. ribisnigri* per plant, but not for their interaction. For alate *N. ribisnigri* a significant effect was seen for host plant only ($F(4,24)= 45$, $p<0.001$). This ANOVA did not include the generated predicted values in the analysis, therefore the WT biotype treatment on *S. arvensis* and *S. oleraceus* were excluded.

As with the previous ANOVA, when considering the number of *N. ribisnigri* (all stages) per plant, the control treatment performed the best with the highest numbers of aphids, and was significantly different to all other hosts except its Rb counterpart.

The only difference resulting from this alternate analysis was that the WT biotype performed significantly better than the Rb biotype on *H. pilosella,* due to the exclusion of *S. arvensis* from the data set.

When considering the counts of alate *N. ribisnigri,* WT *S. arvensis*, WT *S. oleraceus* and Rb *S. oleraceus* treatments were excluded due to zero values. This alternative analysis indicated that the Rb alate biotype on *V. officinalis* had slightly significantly more Rb alates than *S. arvensis*, shown by the predicted means.

Batch two analysis

Batch two consisted of the host plants: *C. intybus, V. arvensis, C. capillaris, V. spicata, S. asper* and *H. aurantiacum.* Figure 5.1.4 shows the transformed means and back-transformed means for the number of alates and all stages of WT and Rb *N. ribisnigri* per plant for each treatment after three weeks. Zero back-transformed values are not presented as they cannot be plotted on a LOG scale.

The ANOVA which took into account the zero values showed that there was no effect of aphid biotype on the number of alates and all stages of *N. ribisnigri,* but there was a significant effect of host plant on both alates ($F(5,20)= 133.85$, $p<0.001$) and all stages ($F(5,44)= 114.82$, $p<0.001$). No interactions were identified between aphid and host plant for either aphid biotype.

When analysing the number of *N. ribisnigri* (all stages), the Rb biotype on *C. intybus* was the best performing treatment with the most aphids per plant, followed by the control treatment. The control treatment was significantly different to Rb and WT biotype treatments on *S. asper, V. spicata* and *V. arvensis.*

WT and Rb *N. ribisnigri* survived on all host plants except *S. asper* which was identified as an unsuitable host plant with zero aphids recorded. *Veronica spicata* could also be considered as an unsuitable host plant as its performance was not significantly different to *S. asper* and it supported very low numbers of both the WT and Rb *N. ribisnigri* biotypes. The analysis suggests that the WT and Rb biotypes performed similarly on each of the host plants screened.

When looking at the number of alate *N. ribisnigri,* the Rb biotype on *C. intybus* had the highest number of alates per plant followed by the WT biotype (control). The

control treatment was significant different to all treatment combinations except the Rb biotype on *C. capillaris.* No differences in recorded alate numbers were observed between the Rb and WT aphid biotypes on each host plant, meaning they performed similarly. No alates were found on *S. asper* for both aphid biotypes and also for the WT biotype on *V. spicata.*

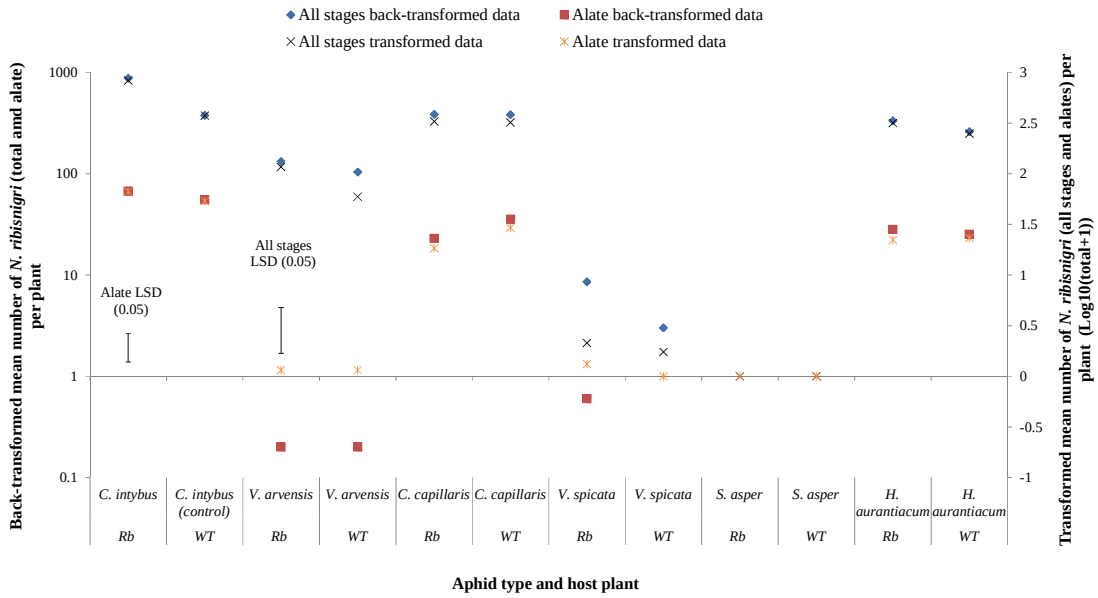


Figure 5.1.4 Transformed and back-transformed mean numbers of alate and all stages of WT and Rb *N. ribisnigri* present after three weeks in Batch two.

For the ANOVA which excluded the zero values and generated predicted values, a significant effect of host plant was identified for alate ($F(4,20)= 53.66$, $p<0.001$) and all stages ($F(4,28)= 33.41$, $p<0.001$) of *N. ribisnigri* per plant. A significant effect of 'aphid' was seen only for total *N. ribisnigri* ($F(1,28)= 8.63$, $p=0.007$) and no interactions were observed between aphid biotype and host plant for alate or all stages of *N. ribisnigri.* This ANOVA did not include the generated predicted means in the analysis for treatments with zero values, therefore the WT and Rb biotype treatments for *S. asper* were excluded.

The only differences resulting from this alternate analysis was that the control was no longer significantly different from the WT biotype on *H. aurantiacum* and the Rb biotype on *C. intybus* was no longer different to the WT biotype on *C. capillaris*.

Furthermore, as a result of the removal of the data set on *S. asper*, the Rb aphid biotype on *V. spicata* became significantly different to both aphid biotypes on *V. arvensis.*

Batch 3 analysis

The data from Batch three did not meet the criteria acceptable for analysis by ANOVA. The mean values are shown in Table 5.1.5. These data confirm that both WT and Rb *N. ribisnigri* cannot survive on *S. arvensis, A. githago, S. vulgaris, C. bursa-pastoris* and *S. media.*

Table 5.1.5 Means numbers of total, alate and non-alate (including nymphs and apterae) *N. ribisnigri* per plant from screening Batch 3 including SE.

Host plant	**Aphid**	**Total *N. ribisnigri***	**Alate *N. ribisnigri***	**Non-alate *N. ribisnigri***
C. intybus	WT	352.8 ± 94.5	26.4± 7.7	326.4± 87.3
S. arvensis	WT	0	0	0
A. githago	WT	0	0	0
S. vulgaris	WT	0	0	0
C. bursa-pastoris	WT	0	0	0
S. media	WT	0	0	0
C. intybus	Rb	562.6± 96.6	40.8± 6.2	521.8± 91.1
S. arvensis	Rb	0	0	0
A. githago	Rb	0	0	0
S. vulgaris	Rb	0	0	0
C. bursa-pastoris	Rb	0	0	0
S. media	Rb	0	0	0

Cirsium arvense and *P. maculosa* had very poor germination rates, so a small preliminary screen was carried out. No aphids of either biotype were recorded on either host plant after three weeks.

5.2. Overwintering host plant experiment

Records were made of the numbers of *N. ribisnigri* and square root transformations were performed, followed by a REML analysis. The fixed model used was date*site*host plant. Predicted means and LSDs could not be calculated for the entire fixed model because some of the host plants used in the sheltered site were not used in the unsheltered sites and there was also a different number of replicates at each site.

The REML indicated a significant effect on the number of *N. ribisnigri* per plant for all fixed terms, which included date (ndf 12, ddf 168, $p<0.001$), site (ndf 1, ddf 11, $p<0.001$), host plant (ndf 3, ddf 10, $p=0.001$), dates*site (ndf 12, ddf 168, $p<0.001$), dates*host plant (ndf 36, ddf, 168, $p=0.026$), site*host plant (ndf 1, ddf 10, $p=0.004$) and dates*site*host plant (ndf 12, ddf 168, $p=0.021$). No effect of the blocking design was observed.

Figure 5.2.2 shows the back-transformed mean number of *N. ribisnigri* per plant present on each sampling date for the host plants sampled from the sheltered and unsheltered sites. WT *N. ribisnigri* survived throughout the winter and were still present in March, with unsheltered *V. arvensis* and sheltered *C. intybus* supporting a mean of 1 and 3.5 aphids per plant respectively on the last sampling date. While these means were small, this study confirms the ability of *N. ribisnigri* to overwinter successfully.

The mean numbers of *N. ribisnigri* per plant recorded throughout the winter varied depending on sampling date. When comparing Figure 5.2.2 with the temperature recordings illustrated in Figure 5.2.3, peak numbers of aphids coincided with peaks in temperature, particularly for *N. ribisnigri* on unsheltered *V. arvensis*. For example, a period of warmer weather occurred from 18 December 2011 until 8 January 2012 and this coincided with an increase in numbers of *N. ribisnigri* on the host plants. These numbers remained high on some weeds until a period of colder weather occurred at the beginning of February, resulting in a sharp decline in aphid numbers.

They then began to increase again but numbers fluctuated with variations in temperature.

Temperature was recorded at the two sites and the box plot in Figure 5.2.4 illustrates the variation in temperature recorded at each site. The unsheltered site experienced the widest variation in temperature and also suffered the lowest temperature. The inter-quartile range for the sheltered site had a smaller range than the unsheltered site, and contains outliers (represented by the green crosses) indicating that the sheltered site experienced higher temperatures compared to the unsheltered site. As the second quartile was not in the centre of the inter-quartile range it suggests that the data required normalisation prior to analysis.

A paired t-test on the mean temperature per day confirmed there was a highly significant difference between the temperatures at the two sites (T= 4.84, df 99, *p*= <0.001). The lowest temperatures recorded were observed on 4 February 2012 at -6.381°C at the sheltered site and -8.365°C at the unsheltered site at 05:11 h and 05:12 h respectively.

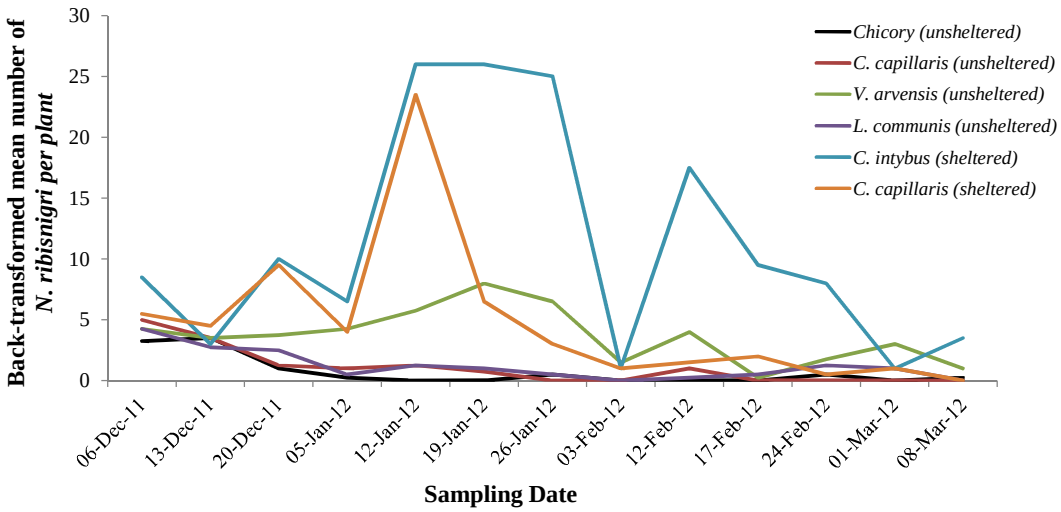


Figure 5.2.2 Back-transformed mean number of WT *N. ribisnigri* for unsheltered *C. intybus*, *C. capillaris*, *V. arvensis* and, *L. communis* and sheltered *C. intybus* and *C. capillaris.*

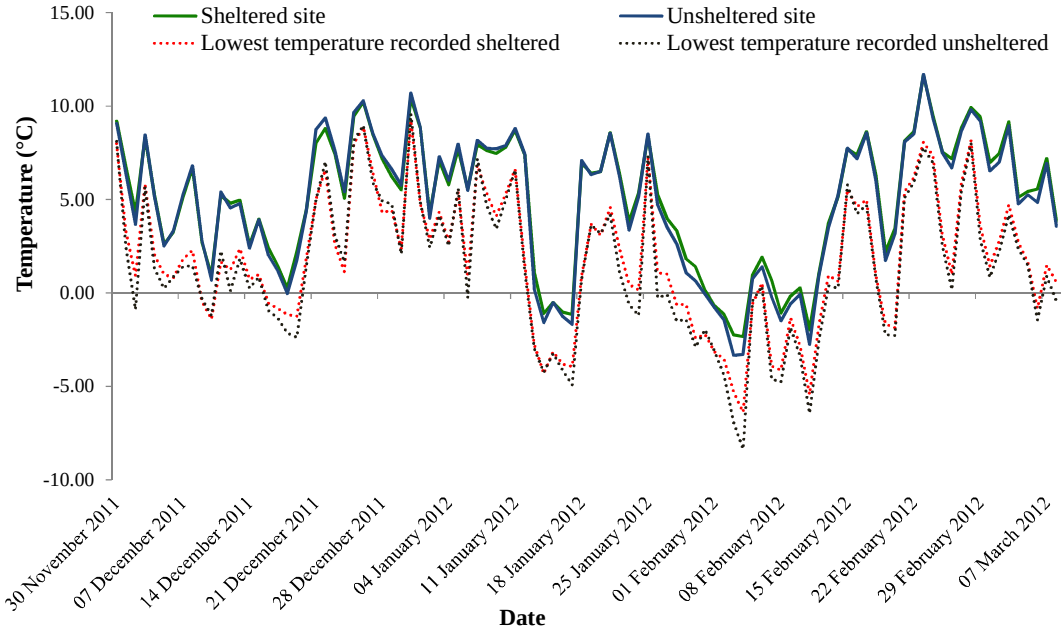


Figure 5.2.3 Mean daily temperatures and lowest recorded daily temperatures (°C) from 30 November 2011 to 7 March 2012 at both sheltered and unsheltered sites.

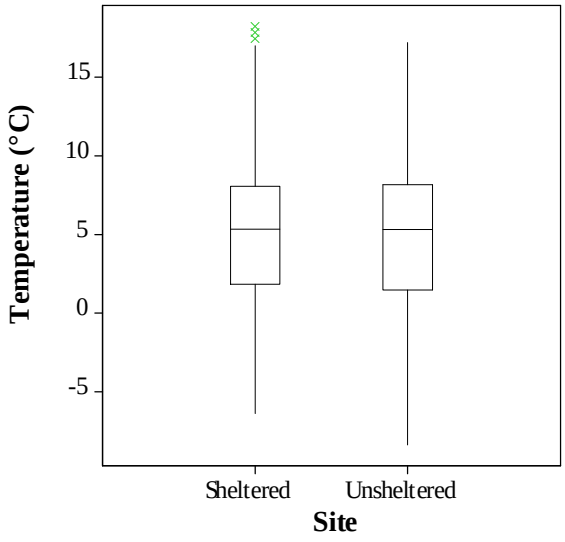


Figure 5.2.4 A box plot to show the temperatures at the sheltered and unsheltered sites. Outlying results are identified by the green crosses.

Both sheltered *C. intybus* and *C. capillaris* supported significantly more aphids per plant than their unsheltered counterparts as shown in Figure 5.2.5. *Chicorium intybus* was the better host plant out of the two and performed better than both the sheltered and unsheltered *C. capillaris.* Observations during the experiment showed there were considerable differences between the quality of sheltered and unsheltered plants, with the sheltered plants growing larger, with superior vigour and healthy green leaves, while the unsheltered plants remained small with drooping dark green foliage and displayed evidence of herbivory.

When comparing the unsheltered host plants, *V. arvensis* was the most suitable overwintering host and was significantly different from all other host plants. *Chicorium intybus, L. communis* and *C. capillaris* performed similarly. *Chicorium intybus* was the poorest overwintering host plant and observations indicated it was a target for other herbivores during the winter, as little plant material was left.

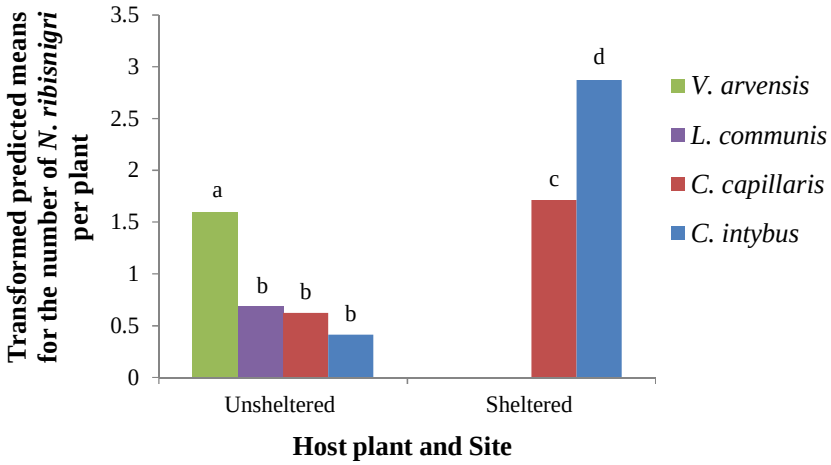


Figure 5.2.5 Predicted transformed means for the number of *N. ribisnigri* using the REML analysis of site*host plant interaction. N.B. Sheltered weeds were only compared with their unsheltered counterparts and not all host plants. Bars with different letters indicate there is a significant difference between mean numbers of *N. ribisnigri* per plant.

Discussion

This study confirmed eight viable host plants for both UK WT and Rb *N. ribisnigri* including *C. intybus, C. capillaris, L. communis, H. aurantiacum, H. pilosella, V. arvensis, V. spicata* and *V. officinalis.* WT and Rb *N. ribisnigri* biotypes demonstrated similar performance on the host plants screened, suggesting that being resistant to Nr-gene lettuce cultivars has had no influence on the Rb biotypes performance on these host plants.

Prior to this study, Blackman and Eastop (2006b) had reported that *S. oleraceus* and *Sonchus arvensis* were suitable hosts for *N. ribisnigri*, but this study does not support this assertion for either WT or Rb *N. ribisnigri*. It was also reported that records existed to support the association of *N. ribisnigri* with *A. githago, C. bursa-pastoris* and *C. arvense,* although low confidence was expressed with regard to the accuracy of these reports (Blackman and Eastop, 2006a). The present study suggests that the aphids found on these host plants were probably misidentified, as they were not suitable hosts. Other unsuitable host plants screened included all the species considered to be very important pest weeds in field vegetable crops (*S. media, C. arvense* and *S. vulgaris*) (HDC, 2009). While this study has identified various unsuitable hosts for *N. ribisnigri*, this may be true only for the vegetative stage of the host plant and not for the flowering stage. For example, research on *B. brassicae* has shown that while it is unable to survive and reproduce on *C. bursa-pastoris* in its vegetative stage, it can do so on flowering heads (Lamb, 1961).

Of all the plant families screened, only those from Scrophulariaceae and Asteraceae contained suitable host plants for *N. ribisnigri.* These two families did, however, also contain species which were unsuitable. Therefore, if several plant species from one family are unsuitable hosts for *N. ribisnigri*, it cannot be assumed that the remaining family members are also unsuitable.

When referring to the orders and families of angiosperms developed by the Angiosperm Phylogeny Group, the Asteraceae (order: Asterales) and Scrophulariaceae (Order: Lamiales) share the same clade, known as asterids, where they are grouped into two further clades known as lamiids and campanulids (APG, 2009). The asterid clade also contains the genus *Lactuca* (Family: Asteraceae), which is the main secondary host of *N. ribisnigri*. It is possible that all other suitable

secondary host plants of *N. ribisnigri* are within the asterid clade. This restriction of host plant choice to particular plant families which are closely-related is a characteristic of many aphid species. However, some highly polyphagous aphids such as *M. persicae* have a much wider host plant range, making *N. ribisnigri* appear quite specialised (Blackman and Eastop, 1984).

Published information on the host plant families of *N. ribisnigri* include the Asteraceae and Scrophulariaceae, as confirmed in this study, but it also includes the Solanaceae (Order: Lamiales), examples of which were not screened (Blackman and Eastop, 2000). As the Solanaceae are members of the lamiid clade of the asterids, it is not surprising that they would provide suitable host plants, being closely taxonomically-related to both the Scrophulariaceae and Asteraceae. This differs from some other insect species which choose plant species which are chemically similar, whilst sometimes being distantly related (Jaenike, 1990). It has been suggested by Peccoud *et al.* (2010) that the restriction of suitable host plants to sets of related plants, as observed for *N. ribisnigri*, demonstrates the strong evolutionary commitment by aphids with regard to their host plant choice.

It is widely accepted that aphids and their host plants have co-evolved throughout history, so that some aphids now utilise a specific range of host plants. There are many hypotheses concerning the factors that have selected for these narrow diet ranges, with the majority focusing on the adaptation of aphid species to particular host plant chemistries, which maximise the performance of their offspring (Levins and MacArthur, 1969; Dixon, 1998;). For example, aphid species do display varying responses to secondary plant compounds (Schoonhoven and Derksen-Koppers, 1976; Niemeyer, 1991) and polysaccharides (Campbell, *et al.*, 1986). The occurrence of host alternation between two often distantly-related plant species, with different secondary compounds and morphology, makes it unlikely that host chemistry is responsible exclusively for plant specialisation (Peccoud, *et al.*, 2010). Furthermore, instances of insects, such as butterflies, not using chemically-suitable host plants, even though they are available, suggests that other factors must be involved (Smiley, 1978).

Often these other factors unrelated to the aphids, such as community structure, act to restrict an aphid's host plant range. It has been hypothesised that insects may adapt

to avoid host plants where their offspring would suffer heavy predation or competition with other species, and therefore adapt to colonise low risk hosts where pressure from natural enemies and competition is low (Smiley, 1978; Jaenike, 1990). For example, white fly (*Bemisia tabaci*) have been observed to learn from past experiences and avoid host plants where offspring have suffered high predation (Nomikou, *et al.*, 2003), and the Peruvian butterfly (*Oleria onega*) selects to lay its eggs in enemy free space away from its host plant to reduce predation (De-silva, *et al.*, 2011). Therefore, while various weed species were suitable host plants for *N. ribisnigri* in this study under laboratory conditions, these results may be different in a field environment as other factors will influence host plant selection i.e. the plant may be avoided due to a high risk of predation. It has also been suggested that host plant chemical composition can have a significant influence on long term aphid-plant associations, with evidence existing to suggest that insects utilise chemically similar host plants which maintains host plant specialisation (Becerra, 1997; Becerra and Venable, 1999).

The role of insect genetics in host plant adaption and preference has been inferred for many years, for instance, where insect populations in the same region express different host plant preferences, although they have access to the same species (Weber, 1985; Schoonhoven, *et al.*, 2005). Host alternation is also thought to be controlled genetically as for some aphid species the alternation is not correlated with patterns in changes of host quality and is thought to be 'programmed' for the selection of the best host plant for mating and oviposition - 'the rendezvous host hypothesis' (Moran, 1983; Dixon, 1998). Following the development of molecular markers, the involvement of insect genetics in host plant selection has now been confirmed by the comparison of aphid host plant races, which adapt through selection to a specific host plant they have utilised for a long period of time, often as a result of geographic variation (Loxdale and Lushai, 2007). For example, the host plant races of *A. pisum* which are adapted to different legume species display different genotypes, with the loci under divergent selection having high genetic variation and being situated close to olfactory receptors and salivary proteins (Jaquiéry, *et al.*, 2012). Ward (1991) suggests that the sympatric divergence of aphids into host races is due to the reproductive isolation resulting from self-fertilisation and anholocycly, rather than assortative mating where mating is more

frequent between aphids with similar genotypes. Specialised adaptations by aphids to specific host plants might include improvements in their ability to manipulate the host and enhance nutritional quality, as observed for *Diuraphis noxia*, *S. graminum* and *A. pisum* (Telang, *et al.*, 1999; Sandström, *et al.*, 2000; Girousse, *et al.*, 2005).

During the present study, the performance of *N. ribisnigri* varied between host plants, indicating that some were more suitable than others. It has been proposed that this could be because such species become less adapted to some host plants as they become more specialised to others (trade-off hypothesis), although evidence supporting this is not consistent (Rausher, 1984; Jaenike, 1990; Mackenzie, 1996; Dixon, 1998). Alternatively, Kindlmann and Dixon (1994) suggested, that if the majority of aphids are located on the host plant that supports optimum performance, then most genetic change and selection would take place on this host, resulting in further adaption and specialisation compared with less preferred hosts. This hypothesis could explain why *N. ribisnigri* appears to be better adapted to utilising suitable host plants belonging to the Asteraceae compared with others as the most genetic change would have taken place on *Lactuca* species (Family: Asteraceae). To support this hypothesis further, it would need to be determined whether *N. ribisnigri* has a preference for *Lactuca sativa* and demonstrates superior performance on this species compared with the other host plants, as while *N. ribisnigri* is observed to colonise lettuce widely in the summer, it has not been confirmed whether they have a preference for lettuce or whether preference for another host is 'concealed' because it is less abundant.

Over the past few years there have been increasing reports that in some temperate regions with mild winters, *N. ribisnigri* has overwintered as a parthenogenetic form. This form may have a selective advantage in areas where the severity of the winter varies and/or there is an absence of the primary host, as with *M. persicae* in Australia (Vorburger, 2004) and *N. ribisnigri* in Central Spain (Nebreda, *et al.*, 2005).

The present study has confirmed that *N. ribisnigri* living on *S. arvensis* in an unsheltered site can survive and reproduce during a winter in central England. The mean temperature was 4.81°C, with a lowest recorded temperature of -8.37°C. This does not mean parthenogenetic forms of *N. ribisnigri* could survive every winter in

this region; the MET Office described the winter of 2011/12 as much milder than the preceding three winters, with temperatures above average (METOffice, 2012).

As expected, the abundance of *N. ribisnigri* in both sheltered and unsheltered sites was closely associated with the changes in temperature during the winter. This type of association has been observed for several species of aphid with anholocyclic life-cycles, where the severity of the winter influences the number of aphids which occur the following year (Harrington and Xia-Nian, 1984; Knight and Bale, 1986; Messina,1993). This is firstly because low temperatures can result in significant mortality, but also because when temperatures are above their lower temperature threshold, aphids can continue to develop, and even reproduce, so that the aphids found in the spring are the descendants of the aphids which entered the winter (Powell and Bale, 2008). In the present study there was a significant effect of sampling date on aphid numbers, where warmer periods led to increases in the numbers of *N. ribisnigri* per plant. While the numbers of aphids per plant were often low in the unsheltered plots, particularly towards the end of the sampling period, it is important to remember that only one fecund aphid survivor is required to found a colony (Powell, *et al.*, 2006).

To survive the winter successfully, aphids can use their ability to cold-harden rapidly and increase their cold tolerance with each generation (Powell and Bale, 2004; Powell and Bale, 2008). Apart from temperature, other factors may influence aphid survival, including leaf wetness, which has been observed to affect the overwintering success of *M. persicae* (Harrington and Xia-Nian, 1984).

In the sheltered sites where *C. intybus* and *C. capilliaris* were planted, only *C. intybus* was a good host for *N. ribisnigri*. When comparing the performance of this host plant in sheltered and unsheltered sites, there was a significant difference in the size and vigour of *C. intybus*, with exposed plants being small in size with wilted leaves and evidence of herbivore attack. It could be inferred that the ability of the host plant to survive and tolerate adverse conditions due to abiotic and biotic factors such as temperature, wind, snow, rain and herbivory may influence the overwintering success of *N. ribisnigri*. For example, in the case of *S. avenae* continued feeding on healthy plant tissue throughout the winter determines

supercooling ability and subsequent survival at freezing temperatures (Knight and Bale, 1986).

While *C. intybus* was the most successful host plant in the laboratory experiments, its poor survival during the winter led to *V. arvensis* becoming a 'better' overwintering host. *Veronica arvensis* was the only exposed weed which maintained *N. ribisnigri* until the final monitoring date in March. This may be due to its capacity to overwinter, particularly when compared with the other host plants and also to its morphology, since its dense foliage and complex branching structure undoubtedly provided shelter for *N. ribisnigri*, as illustrated in Figure 5.3.

It is likely that the microclimate within the foliage of *V. arvensis* varied from ambient conditions and that the leaf boundary layer provided more favourable conditions in terms of temperature and humidity (Schoonhoven, *et al.*, 2005). Similar advantages of certain overwintering sites were observed in a study on *D. noxia*, where survival was longest on wheatgrass, where the compact tussocks were thought to provide shelter from cold injury and desiccation (Messina, 1993). *Sitobian avenae* crawls into the centre of hedgerows and grass tussocks (Leather, *et al.*, 1993).When compared with *V. arvensis*, the other host plants in the present study had less foliage and a greater surface area was exposed to the winter elements. It was also clear during monitoring that herbivores utilised almost all of the host plants, with the exception of *V. arvensis* and the plant did not have to respond to this additional stress.

Figure 5.3 *Veronica arvensis* (left) and *N. ribisnigri* within its branching structure (right).

In conclusion, this study has identified host plants which are potential sources of overwintering *N. ribisnigri* in sheltered and unsheltered environments. It has confirmed that *N. ribisnigri* can overwinter as adults/nymphs, and with the occurrence of very cold winters expected to become increasingly rare as a result of climate change, selection for anholocyclic clones of *N. ribisnigri* is likely to increase (Collier, 2007b). As a result, this could lead to higher numbers of *N. ribisnigri* infesting lettuce earlier in the summer, as the number of aphids leaving overwintering hosts is no longer restricted to the number of eggs laid in the previous autumn; parthenogenetic aphids can begin reproducing as soon as spring temperatures are suitable for development (Leather, *et al.*, 1993).

Thus if warmer winters were to favour the overwintering of active stages, weed management during the winter might be one option for control. However, the environmental impact of the destruction of these weed hosts would need to be investigated. Other future work could consider overwintering success on other host plants, in addition to *C. intybus* and *C. capillaris*, in a range of sheltered environments. In addition, an investigation on overwintering survival on more established and older host plants might provide different results.

Conclusion

- This study confirmed that eight species of plant (mainly wild species) are suitable hosts for both UK WT and Rb *N. ribisnigri*. This includes *C. intybus, C. capillaris, L. communis, H. aurantiacum, H. pilosella, V. arvensis, V. spicata* and *V. officinalis*.
- The performance of WT and Rb *N. ribisnigri* biotypes was similar regardless of the host plant.
- *Nasonovia ribisnigri* (WT4850a) survived and reproduced during a winter in central England. The results indicate that abundance will be determined by the suitability of the host plant and the severity of the winter.

Chapter 6: Population Dynamics

Introduction

Aphids display strong seasonal cycles of abundance, consisting of rapid outbreaks and local extinctions, which are regulated by both extrinsic (host plant availability, predation, weather) and intrinsic factors (competitive ability, emigration, resistance to predation and development rates) (Karley, *et al.*, 2004; Schowalter, 2006).

The long term population dynamics of pest aphids, such as *N. ribisnigri*, on agricultural crops, such as lettuce, are challenging to study due to the short growing season of their host plants and the variation in both abundance and distribution of the crop each year (Kindlmann, *et al.*, 2007). As a result, the majority of studies have concentrated on tree-dwelling aphids which can be studied continuously over a period of several years (Dixon, 1998). Collectively, these studies have outlined the main characteristics of aphid development and population dynamics throughout the year.

Generally, in response to the high levels of nutrition provided by spring host plants, aphids display an increase in population size, which is followed by a sharp decline during summer, and for some species a period of recovery resulting in a second peak during autumn (Kindlmann, *et al.*, 2007). Aphids then survive the winter in lower numbers in the form of a diapausing egg or as an anholocylic clone (Phillips, *et al.*, 2000).

For some researchers, the most interesting observation is the mid-summer decline, which has been observed in various aphid species including the *A. pisum*, *M. persicae* and *M. euphorbiae* (Losey and Denno, 1999). In the UK, this occurs in mid-July and is referred to as the 'mid-summer crash', where populations decline rapidly, remaining low for up to six-eight weeks (Tatchell, *et al.*, 1998; Karley, *et al.*, 2004). As it is also observed in non-crop aphid/host plant systems, such as *Myzocallis boerneri* on Turkey oak trees (Jarosik and Dixon, 1999), this decline is not restricted solely to agricultural crops and their related cropping practices such as harvesting and insecticide application (Karley, *et al.*, 2003). Between 1994 and 1996, work carried out at Warwick HRI (now Warwick Crop Centre) showed that *N.*

ribisnigri populations declined during mid-July to early September, with aphids being abundant both before and after this period, with the second peak being the largest (Collier, *et al.*, 1999).

Considerable research has been carried out to try to understand the factors which cause the mid-summer crash since manipulation of environmental or other conditions within a crop could provide potential new methods of aphid control. For population declines such as this to occur, one would expect to see either 1) an increase in mortality, 2) decreased fecundity or 3) mass emigration, all of which can be caused by a multitude of intrinsic and extrinsic factors, including weather, host plant quality and natural enemies (Karley, *et al.*, 2003).

Weather conditions

Temperature has a significant effect on the development and reproduction of aphids as discussed in Chapter 3, and weather conditions will naturally influence population growth. Lowe (1966) provided evidence of this through observations of *B. brassicae*, where seasonal population changes mirrored changes in temperature. Other studies have shown that when temperatures have been unusually high, population crashes have occurred subsequently (Palumbo, 2000a; Palumbo, 2000b), even when other factors regulating populations, such as aphid predators, have been excluded (Basky, 1993). This suggests that high temperatures could be regulating aphid populations, either alone or in combination with other factors.

When considering other weather variables, differences between years in autumn and spring survival rates of *R. padi* have been negatively correlated with rainfall (Lowe, 1966), and aphid populations on other crops have been wiped out by heavy precipitation and strong winds (Jones, 1979; Hughes, 1963). A study on the Turkey oak aphid, however, showed that when aphid populations are large like they are prior to the mid-summer crash, weather conditions such as temperature, wind and precipitation do not regulate population size significantly (Jarosik and Dixon, 1999).

With regard to the mid-summer crash, periods of high temperature and severe rainfall do not provide a consistent explanation for the yearly occurrence of the crash in different localities (Karley, *et al.*, 2004). Therefore, this suggests that weather variables are more likely to be interacting and influencing other factors than being

the major cause of the mid-summer crash, including effects on natural enemies and host plant quality. Recent field observations on three species of potato aphid indicated that populations were regulated by weather factors only through their indirect influence on natural enemies (Alyokhin, *et al.*, 2011).

Host plant quality

The quality of a host plant can be affected by a range of factors which can indirectly influence the performance, distribution and abundance of an aphid infestation by altering the suitability of the host plant through changes in plant nutrients and metabolites (Douglas, 2003). In response to the change in the seasons, plants naturally vary in their nutritional content (amino acid composition), water content and allelochemicals, and aphid abundance is known to reflect this, with aphids generally being present in high numbers on plants in spring when their nutritional value is higher, compared with mature crops which are less favourable nutritionally (Dixon, 1987a; Dixon, *et al.*, 1993b; Karley, *et al.*, 2002; Schoonhoven, *et al.*, 2005). For example, *B. brassicae* populations were observed to increase on new host plants until the host plant condition deteriorated, where a decline in the population then occurred, which is often exacerbated by other extrinsic factors (Hughes, 1963).

Various studies have shown changes in aphid performance and abundance in response to changes in phloem nutrients, but studies investigating these changes at a chemical level, specifically in relation to the mid-summer crash, are limited. A single study by Karley *et al.* (2003), found that the changes in amino acid composition in the phloem of potato leaflets (decline in glutamine) during development consistently coincided each year with the beginning of the crash. However, these changes could not be confirmed as being exclusively accountable.

In addition to seasonal changes, other extrinsic stress factors can influence host plant quality, including the presence of other herbivores, water availability and humidity, all of which can indirectly affect aphid populations. For example, prolonged drought can reduce aphid feeding through reduced plant turgor and increased sap viscosity, while intermittent drought may make plants more suitable by increasing the availability of amino acids and reducing plant defences which would favour insect development (Crawley, 1983; Mattson and Haack, 1987). Furthermore, different

aphid species display varying responses to drought, where for example, water-stressed barley resulted in higher densities of *D. noxia*, while *R. maidis* densities were lower (Oswald and Brewer, 1997).

When considering the influence of herbivores on their host plant, prior to the mid-summer crash, aphid populations are at their peak and intra-specific competition for nutrients is high, therefore affecting plant quality. As a result, aphid performance is affected, with longer development times, reduced fecundity, smaller aphids and an increase in the number of alate morphs to colonize new resources (Dixon, 1998). With reduced reproduction and increased emigration this would assist and intensify the observed mid-summer decline. Therefore, factors influencing host plant quality should not be overlooked, as while their influences may not be present during every mid-summer crash, they could be a contributing factor in some years.

Natural enemies

Natural enemies are responsible for the top down control of aphid populations and various studies have reported significant regulation of aphids by natural enemies and fungal disease (Nielsen and Hajek, 2005; Hirose, 2006; Alyokhin, *et al.*, 2011). Aphids are infected by various entomopathogenic fungi of which the majority are Entomophthorales (Zygomycota), with the most common species being *Pandora neoaphidis*, *Neozygites fresenii* and *Entomophthora planchoniana* (Steinkraus, 2006). Key obligatory predatory and parasitic insects include ladybird adults and their larvae (Coleoptera: Coccinellidae), lacewing adults and their larvae (Neuroptera: Chrysopidae and Hemerobiidae), hoverfly larvae (Diptera: Syrphidae), parasitoid wasps and predatory bugs such as flowerbugs (Hemiptera: Anthocoridae) (Rotheray, 1989). Other facultative predators include earwigs (Dermaptera: Forficulidae), ground beetles and rove beetles (Coleoptera: Carabidae and Staphylinidae).

A vast number of studies have been carried out on the natural predation of aphids and these have recognised the Coccinellidae as one of the most important insect families in controlling aphids (Obrycki and Kring, 1998; Wyss, *et al.*, 1999; Rutledge, *et al.*, 2004). As predator communities vary between years, it is more likely that a combination of predators from more than one family contribute to the

control of aphids (Alyokhin, *et al.*, 2011). For example, parasitoids have also been identified to be important natural enemies in regulating aphid populations, as they have been observed to display peaks in numbers followed by a decline in aphid numbers (Müller, *et al.*, 1999). Furthermore, there is evidence that maximum pest suppression results from a number of species of natural enemy and not just one, as pest suppression is lower when the predicted summed effects of each natural enemy alone are calculated (Cardinale, *et al.*, 2003).

Generally, determining the effect of predators on aphid populations is difficult to assess as they do not always leave traces of the prey, in contrast to the mummies and infection left by parasitoids and pathogens (Tamaki and Weeks, 1973). As a result, predator exclusion experiments are often performed to compare aphid populations in the presence and absence of predators. However, these results need to be interpreted carefully as various studies have identified changes in the microclimate (humidity, temperature, light intensity and wind) and predator/prey behaviour in caged experiments when compared to un-caged areas (Luck, *et al.*, 1988). Various researchers have tried to improve exclusion techniques by using varying mesh sizes and cage structures, while others have opted for other methods such direct field observation or predator removal (Costamagna and Landis, 2007; Meihls, *et al.*, 2010). Luck *et al.* (1988) have provided a detailed review of the different methods which can be used for evaluating the impact of natural enemies.

During the period of the mid-summer crash, exclusion studies have shown declines in aphid numbers which coincide with an increase in predator numbers, leading the authors to propose that predators are the most important factor reducing aphid populations (Nunnenmacher and Goldbach, 1996). Generally, predator exclusion studies report higher rates of increase and total numbers of aphids when natural enemies are excluded; indicating they play a significant role in reducing or regulating populations (Meihls, *et al.*, 2010).

When considering the potential role of fungal diseases in the mid-summer crash, various studies have shown declines in aphid populations with the increase and presence of entomopathogenic fungi (McLeod, *et al.*, 1998; Plantegenest, *et al.*, 2001). Populations of *Aphis glycine* have been observed to crash following an epizootic, and infection levels were density dependent being more prevalent when

aphid populations were higher (Nielsen and Hajek, 2005). Investigations into the use of entomopathogenic fungi as biological control agents for the control of aphids have been undertaken, with the inoculation of *Pandora neoaphidis* and *Neozygites fresenii* causing early season crashes in *A. fabae* in two of the four years of trials (Wilding, 1981). However, many other release attempts have been relatively unsuccessful (Powell and Pell, 2007).

While natural enemies and entomopathogenic fungi do appear to have a significant effect on aphid populations, their success is again indirectly affected by other factors, particularly the host plant. Various studies have recognised the importance of plant genotypes in indirectly influencing the distribution and abundance of natural enemy populations by directly affecting aphid population growth rates (Johnson, 2008; Schädler, *et al.*, 2010). Furthermore, differences in plant morphology can determine the success of predation by differentially affecting searching by predators and parasitoids. A study comparing predation success between normal and leafless peas, showed that coccinellids were more effective on leafless varieties (Kareiva and Sahakian, 1990), while a more recent study showed more effective predation by lady beetles and lacewings on highly-branched pea varieties (Reynolds and Cuddington, 2012). For entomopathogenic fungi, the presence of leaf waxes has been shown to affect the level of infection, with infection of *A. pisum* by *P. neoaphidis* being greater on a variety with reduced surface wax bloom (Duetting, *et al.*, 2003). With regard to lettuce morphology, the formation of heads can protect lettuce aphids from natural enemies and alter the microclimate, influencing the behaviour of pathogens and possibly making the aphids more vulnerable to fungal infection (Nunnenmacher and Goldbach, 1996).

This effect of the microclimate again demonstrates the influence that weather conditions could have on natural enemies, by not only favouring their development, but by improving their predation and reproduction efficiency, as observed for coccinellids in warmer climates (Frazer, *et al.*, 1981a). Temperature, humidity and light also influence the oviposition behaviour of hover flies which influences the success of their progeny as predators (Tenhumberg and Poehling, 1995). For fungi, the indirect effects of precipitation, and the resulting increase in humidity, could influence incidence, particularly as different species have varying temperature

optima and humidity requirements (Steinkraus, 2006; Alyokhin, *et al.*, 2011). However, some studies have found no relationship between infection and weather conditions (Wilding and Perry, 1980).

Mass emigration

Aphids move between host plants and also migrate in response to overcrowding and hosts that are deteriorating nutritionally (Groeters, 1989). As aphid numbers increase, the production of alate forms also increases, and this is well demonstrated by *A. pisum*, where the density of migrants in suction traps is positively related to the size of crop infestations (McVean, *et al.*, 1999). The development of a mechanistic model has suggested that density dependent dispersal could be the sole cause of the decline for the alder aphid (*Pterocallis alni*) and other species (Mashanova, *et al.*, 2008).

Stimulation of alate production can also occur in response to predation, through detection of alarm pheromone or subsequent movement and crowding (Le Ralec, *et al.*, 2010). Therefore, it is reasonable to consider increased emigration as a cause of the mid-summer crash, in response to high numbers of aphids and natural enemies. However, field studies often indicate that numbers of alates are low (<10%) prior to a crash, and if aphids were emigrating from deteriorating plants, or escaping predation, one would expect re-colonisation of nearby host plants, which has not been observed (Karley, *et al.*, 2003).

Thus, the results of previous studies demonstrate the complexity of aphid population dynamics which can be influenced by a range of density dependent and density independent factors. This study aims to determine the role of natural enemies and entomopathogenic fungi in regulating *N. ribisnigri* populations with a particular focus on the mid-summer crash.

Methods

6.1 Field trial 2010

This preliminary experiment took place between June and October 2010 at Warwick Crop Centre, Wellesbourne in a field known as Sheep Pens (National Grid Reference SP 26919 56652). The purpose of this experiment was to develop techniques to determine the effects of entomopathogenic fungi and arthropod predators on the development of *N. ribisnigri* populations in the field, with a particular focus on the mid-summer crash.

The field trial consisted of nine treatments which combined various fungicide, insecticide and netting regimes (see Table 6.1.1). There were two replicates of each treatment (18 plots in total) in each trial and the trial was repeated on three occasions during the summer to allow continuous observation of *N. ribisnigri* populations.

The fungicide, insecticide and netting treatments were used in combination or individually to reduce/exclude aphid natural enemies and/or entomopathogenic fungi. Netted treatments (Enviromesh® 1.35mm) were used to allow aphids to develop undisturbed by restricting predator access. A broad spectrum pyrethroid insecticide with contact and residual activity was applied at 0.3L/ha (Decis®- deltamethrin) to reduce the occurrence of aphid natural enemies. This was reported to have low toxicity to predatory ground beetles, lacewings, parasitized aphids, low residual toxicity to parasitic wasps, moderate toxicity to ladybirds, and high toxicity to hoverfly larvae (Bayer, 2011). A broad spectrum fungicide was applied at 0.4kg/ha (Nativo®- trifloxystrobin + tebuconazole) to reduce the occurrence of entomopathogenic fungi. These active ingredients were selected following a literature review of fungicides which negatively affected entomophthorales.

One study which was reviewed showed a significant reduction in epizootics of *P. neaoaphidis* on *A. glycines* from strobilurin and triazole mixes (including tebuconazole and pyraclostrobin; pyraclostrobin and trifloxystrobin) (Koch, *et al.*, 2010). A second study screening 20 fungicides, showed that chlorothalonil, fenipropimorph, spiroxamine and tebuconazole inhibited *Erynia neoaphidis* infectivity (Latteur and Jansen, 2002). Of these potential candidates to be used in the present study, chlorothalonil was excluded, as a study by Wells *et al.* (2000) found

it did not stop infection of *A. gossypii* by *N. fresenii* . As a result of this review, tebuconazole was selected as a suitable active ingredient to be used in this study. A product was sought which contained tebuconazle and either fenipropimorph, spiroxamine or a strobilurin (preferably pyraclostrobin and trifloxystrobin) which also appeared to have the desired effect. The product Nativo® was selected as suitable candidate as it contained both trifloxystrobin + tebuconazole with no concerns about phytotoxic effects on lettuce.

Table 6.1.1 Nine treatments included in the 2010 field trial with various fungicide, insecticide and netting regimes (F = fungicide; I = insecticide).

Treat. num.	Treat. name	Netting	F treatment	I treatment	Infested artificially
1	Netted	Yes	No	No	Yes
2	Open	No	No	No	Yes
3 Control	Control	No	No	No	No
4	Netted+F	Yes	Yes	No	Yes
5	Open+F	No	Yes	No	Yes
6	Netted+I	Yes	No	Yes	Yes
7	Open+I	No	No	Yes	Yes
8	Open+F+I	No	Yes	Yes	Yes
9	Netted+F+I	Yes	Yes	Yes	Yes

Eight hundred seeds (cv. Saladin Supreme (untreated) were sown in peat blocks on 11 May, 16 June and 20 July. The lettuce plants were grown in a glasshouse and transplanted after approximately four weeks of growth when their $4^{th} – 5^{th}$ true leaf had unfolded (BBCH growth stage 14 or 15). One week before transplanting, plants were transferred to a cold frame to harden off. The lettuce plants were scheduled to be transplanted on 9 June, 15 July and 18 August respectively into plots (one bed= 1.83 x 3.5m) containing 40 plants (4 x 10 @ 35cm spacing). However, the transplanting scheduled for 15 July took place on 19 July because high winds prevented spraying, and the transplanting due on 18 August took place on 31 August following a significant period of rainfall, which made conditions too wet for ground

preparation. These two batches of lettuce plants were transferred to lower temperatures prior to transplanting to delay growth.

Fungicide, insecticide and netting (Enviromesh® 1.35mm) treatments were applied on the same day as transplanting. Treatments with no netting were protected from birds using wider mesh netting. The treatments were arranged in a 3 x 3 randomised split plot design which was different on each of the three field trial occasions. The control was always situated in the centre of the design, with the open treatments in each corner, to limit movement of *N. ribisnigri* from untreated plots into the control. Figure 6.1.2 shows the layout of the field trials.

Figure 6.1.2 Image taken of 2010 field trial (June occasion).

The day after transplanting, 15 plants in each plot (except the control treatments) were inoculated with five wingless adult (or 4th instar) aphids of clone WT4850a (See Figure 6.1.3). The aphids had been placed in Eppendorf® tubes over the two preceding days and were stored in a refrigerator to prolong their survival. Although it would have been preferable to monitor a natural infestation of *N. ribisnigri* in all of the field trials, their occurrence could not be relied upon.

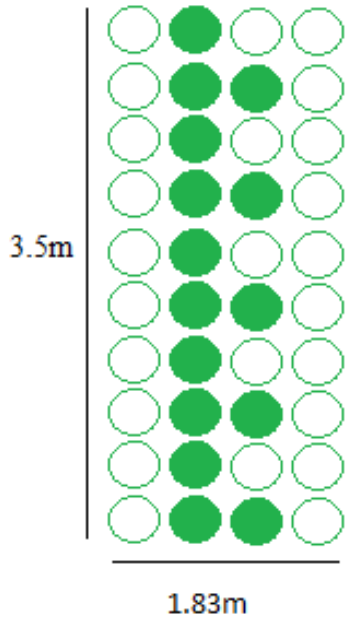


Figure 6.1.3 Field plot (1.83 x 3.5m) containing 40 plants (4 x 10 @ 35 cm spacing). Closed circles indicate plants inoculated with five adult (or 4th instar) *N. ribisnigri* (WT4850a).

Each week, over a period of five-six weeks, four plants were sampled from each bed/treatment (72 plants per week). Samples were removed from alternate ends of each bed each week to maintain bed integrity and were stored in labelled paper bags in a cold store at 5°C D:D. Whole plants were sampled and examined until they grew to an unmanageable size and then only half of each lettuce plant was examined.

Weekly sampling dates varied depending on weather conditions. Plants from occasion one were sampled on 18, 25 June, 5, 12, 19 and 27 July. Plants from occasion two were sampled on 28 July, 3, 11, 18, 24 and 31 August. Plants from occasion three were sampled on 8, 15, 21, 28 September and 7 October.

Plants were sampled destructively and the numbers of aphids and natural enemies were recorded including Coccinellidae, Araneae, Anthocoridae, Neuroptera, syrphid larvae and parasitized aphids. All insects were identified to family, and where possible to species except for syrphid larvae. The level of parasitism was estimated through the number of parasitized aphids but because the aphid species which were parasitized were not identified, the data only represented overall aphid parasitism.

Due to the amount of data collection involved in this experiment, entomopathogenic fungi were not assessed. All insects were stored in 70% ethanol in case further identification was required.

6.2 Field trial 2011

Experimental plot

This experiment took place during two weeks of each month from May-September 2011 (five occasions) at Warwick Crop Centre, Wellesbourne in a field known as Big Cherry (National Grid Reference SP 27354 56153). As outlined in Table 6.2.1, the field trial consisted of nine treatments, which included fungicide or insecticide treatments combined with three netting regimes. There were two replicates of each treatment (18 plots in total).

As with the field trial in 2010, fungicide and insecticide applications were used to reduce the numbers of natural enemies and/or entomopathogenic fungi. The treatment where insecticide and fungicide were combined was removed, as no effect was seen during the trial in 2010. Instead, the following three netting regimes were introduced:

- Open - beds open for the entire two week experimental period allowing the movement of natural enemies in and out of the plots.
- Permanently netted - beds permanently netted for entire two week experimental period to exclude natural enemies.
- Temporarily netted - beds netted for the first week of experimental period and then uncovered to allow natural enemies to move into the plots.

It was assumed that, after one week, the populations of *N. ribisnigri* in the permanently netted plots would be equal to those in the temporarily netted plots, since both had been covered for one week. By uncovering the temporarily netted beds after one week, the effect of introducing natural enemies could be determined by comparing the numbers of *N. ribisnigri* at the end of the two week experimental period with the numbers present in the permanently netted and open plots.

Table 6.2.1 Nine treatments included in the 2011 field trial including fungicide or insecticide application combined with different periods of netting (F= fungicide and I= insecticide).

Treat. num.	Treatment name	Period netted for	F application	I application
1 (Control)	Open	Never	No	No
2	Open+F	Never	Yes	No
3	Open+I	Never	No	Yes
4	Temp netted	1 week	No	No
5	Temp netted+F	1 week	Yes	No
6	Temp netted+I	1 week	No	Yes
7	Perm netted	2 weeks	No	No
8	Perm netted+F	2 weeks	Yes	No
9	Perm netted+I	2 weeks	No	Yes

When compared with the field trial in 2010, shortening the trial period to two weeks provided a snap shot of the effect of natural enemies in each month and also ensured that the insecticide and fungicide treatments remained more effective over the two week period. Furthermore, shorter sampling periods made data collection more manageable since the plants were smaller.

During the 2010 trial, there were no observed effects of either the Decis® or Nativo® applications. As a result in 2011, the broad spectrum fungicide Amistar® was applied (1L/ha) instead of Nativo. This is a systemic, translaminar and protectant strobilurin fungicide (Azoxystrobin). Decis was used again (0.3L/ha.), since a greater effect might be observed by having a shorter trial period.

Four hundred seeds (cv. Saladin Supreme (untreated)) were sown in peat blocks on 12 April, 16 May, 13 June, 18 July and 15 August and these were scheduled for transplanting on 9 May, 14 June, 12 July, 15 August and 12 September respectively. However, the September transplanting was delayed until 15 September to fit around other experimental commitments.

The plants were grown in a glasshouse and transplanted after approximately four weeks of growth when their 4th - 5th true leaf had unfolded. Plants were transplanted into 18 plots (one plot= 1.83 x 3.5m) of 20 plants (4 x 5 @ 35cm spacing). One week before transplanting, plants were moved to a cold frame to harden off. Fungicide, insecticide and netting treatments were applied on the same day as transplanting. The treatments were arranged in a 3 x 3 block design which was randomised for each occasion. Treatments which were open were protected from bird damage with wide mesh netting. The experiment was located close to hedgerows containing wild flowers to increase the proximity of natural enemies. Figure 6.2.2 shows the trial planted on 9 May.

Figure 6.2.2 Field trial in 2011 (planted 9 May 2011).

The day after transplanting, ten plants in each bed were inoculated with five wingless adult (or 4th instar) aphids of clone WT4850a. These were placed in Eppendorf® tubes during the preceding two days and stored in a refrigerator to prolong their survival. Figure 6.2.3 identifies the lettuce plants which were inoculated.

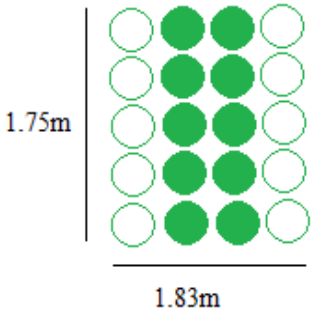


Figure 6.2.3 Field plot (1.83 x 1.75m) containing 20 plants (4 x 5 @ 35cm spacing). Closed green circles indicate plants inoculated with five adult (or 4th instar) *N. ribisnigri* (WT4850a).

One week later, the netting was removed from six of the temporarily netted plots (treatments 4-6 in Table 6.2.1 for both replicates) and the plots were protected with bird netting. This was done on 17 May, 22 June, 19 July, 23 August and 23 September. One week later, eight plants were sampled from each plot. These were chosen at random by selecting eight numbers between 1-20 which corresponded to the plants in the plot. Figure 6.2.4 indicates the eight plants sampled from each plot, of which half were infested artificially with *N. ribisnigri*. Sampling took place on 24 May, 29 June+1 July, 26 July, 30 August and 29-30 September, which was usually over a period of one day, weather permitting.

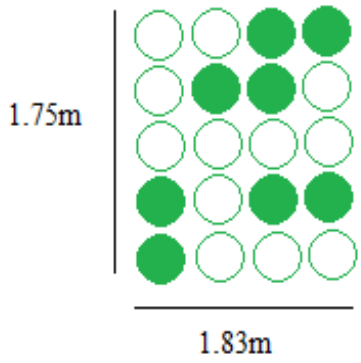


Figure 6.2.4 Field plot (1.83 x 1.75m) containing 20 plants (4 x 5 @ 35cm spacing). Closed green circles indicate the plants sampled from each plot.

Whole plants were sampled destructively and aphids and natural enemies were identified, counted and recorded. Natural enemies were recorded and stored as in 2010. Aphids infected with entomopathgenic fungi (Figure 6.2.5) were also counted, and recorded as either an early-middle infection (fungal mycelia/branching emerging from the aphid body) or late infection (swelling and discoloration of the body (often described as ‘creamy’ and ‘snotty’). The species of aphid infected by entomopathogenic fungi were not determined but the sampling did show the general level of infection by entomopathogenic fungi.

Figure 6.2.5 Early-middle (left) and late (right) stage entomopathogenic fungal infections of *N. ribisnigri.*

Tinytag© loggers were used to record the ambient temperature and humidity every 30 minutes. A logger was placed in the centre of a permanently netted and an open plot to determine the effect of netting. Yellow water traps were placed in the empty beds between plots for the two week trial period to sample aerial insects and were emptied approximately once a week.

Monitoring plot

In addition to the monthly trials, a monitoring plot was established in a separate area of the same field to allow the build-up of a *N. ribisnigri* infestation (infested artificially) where the development of an infestation and the timing of the mid-summer crash could be monitored. One hundred seeds (cv. Saladin Supreme (untreated)) were sown in peat blocks on 12 April, 5 May, 23 May, 13 June, 4 July, 25 July and 15 August and transplanted on 10 May, 31 May, 20 June, 12 July, 1 August, 22 August, 15 September respectively. Plants were grown in the glasshouse and after approximately four weeks of growth, they were transplanted into the field in two plots (one plot= 1.83 x 3.5m) containing 40 plants (4 x 10 @ 35cm spacing). Every three weeks, a further pair of plots was added behind the existing plots until 12 September, to provide 'temporally overlapping' plots containing plants of a range of ages. The monitoring plot was surrounded with an electric fence. Figure 6.2.6 shows the monitoring plot planted on 9 May.

The day after transplanting, 15 plants in each plot were inoculated with five wingless adult (or 4th instar) aphids of clone WT4850a as per Figure 6.1.3. These had been

placed in Eppendorf® tubes over the preceding two days and stored in a refrigerator to prolong survival. Four plants were sampled from each bed on 25 May, 8, 14, 20 June, 9, 14, 22, 29 July, 16, 23, 30 August, 5, 15, 23 September, 4, 12, 19 and 26 October. Each bed was sampled for up to five weeks depending on plant quality, and samples were removed from alternate ends of each bed to maintain bed integrity. Sampled lettuce plants were stored in labelled paper bags in a cold store at 5°C D:D. Whole plants were sampled destructively and the numbers of aphids, natural enemies and aphids infected were identified, recorded and stored as per the previous field trials. Yellow water traps were placed in between the plots and sampled every week.

Figure 6.2.6 Monitoring plot in 2011 (9 May 2011).

Statistical analysis

6.1 Field trial 2010

The total number of aphid stages and natural enemies recorded on the lettuces sampled from each plot was determined. A mean per plant was then calculated by dividing the totals by the number of lettuces sampled.

An ANOVA was performed on the numbers of alate, non-alate (including nymphs and apterae) and all stages of *N. ribisnigri* and natural enemies including; Coccinellidae, Araneae, syrphid larvae, parasitized aphids, Anthocoridae and Neuroptera. As natural enemy numbers were low, the counts of larvae and adults

were combined for Coccinellidae and Neuroptera (Chrysopidae and Hemerobiidae). Data on some of the aphid-specific natural enemies were also combined to analyse the effect of treatments on total predator numbers (including: Anthocoridae, Coccinellidae, Neuroptera, parasitized aphids and syrphid larvae).

The ANOVA was performed using the blocking structure: occasion/replication/sampling_week, where occasion represented the three field trials (planting date), replication represented the two replications of each of the treatments and sampling week represented the five sampling weeks on each trial. The treatment structure used included: sampling_week*(netting*fungicide*insecticide) which contrasted the means for the netting, fungicide and insecticide treatments for each sampling week. The control treatment was not included in the ANOVA as these aphids were from a natural infestation making this treatment unsuitable for comparison with those treatments which were inoculated. Therefore, when interpreting the results, all treatments were compared to the open plot with no insecticide/fungicide treatment, which was essentially the inoculated control.

Due to the large number of zero values present in the dataset, a value of one was added to all data and LOG values were used to normalise the data. The LOG value was then divided by the number of plants sampled from that treatment, to provide a proportion of the data variable per plant. This was because different numbers of plants were sampled from each treatment with sometimes only half a plant being destructively sampled.

In addition to the ANOVA, the relationships between the recorded variables (natural enemies, *N. ribisnigri* (alate, apterous and nymphs) were determined using scatter plots. Where linear relationships were observed, Pearson R correlations and linear regressions were also performed for each sampling date during the mid-summer crash with the data grouped into netted and open (i.e. combining fungicide, insecticide treatment data) to determine any relationship which might explain the aphid decline.

6.2 Field trial 2011

Only data collected from plants which were infested artificially with *N. ribisnigri* were included in the analysis as plants that were not infested artificially had significantly lower numbers of aphids. Only data from the experimental plots were analysed with the ANOVA (not the monitoring plot).

An ANOVA was performed on the numbers of alate and all stages of *N. ribisnigri*, parasitized aphids and aphids infected with entomopathogenic fungi. Coccinellidae, Anthocoridae and syrphid larvae were present in very low numbers (not exceeding a mean of one), resulting in zero values for the majority of the treatments and no Neuroptera were recorded. Therefore, ANOVAs were not performed on these variables due to the lack of data. Anthocoridae, Coccinellidae, parasitized aphids and syrphid larvae were, however, summed together to analyse the total number of natural enemies.

The ANOVA took into account the blocking structure: field trial/replication, where field trial represented the five field trial occasions (planting date) and replication represented the two replications of each of the treatments. The treatment structure included planting date*netting*treatment. Due to the numbers of zero values present in the dataset, a value of one was added to all data and a LOG transformation was made to normalise the data.

Relationships between the recorded variables were determined using scatter plots. As described for Field Trial 2010, Pearson R correlations and linear regressions were performed when linear relationships were observed. This process was performed on the data from the artificially infested plants only, for each individual field trial, with the data grouped by netting treatment.

Results

6.1 Field trial 2010

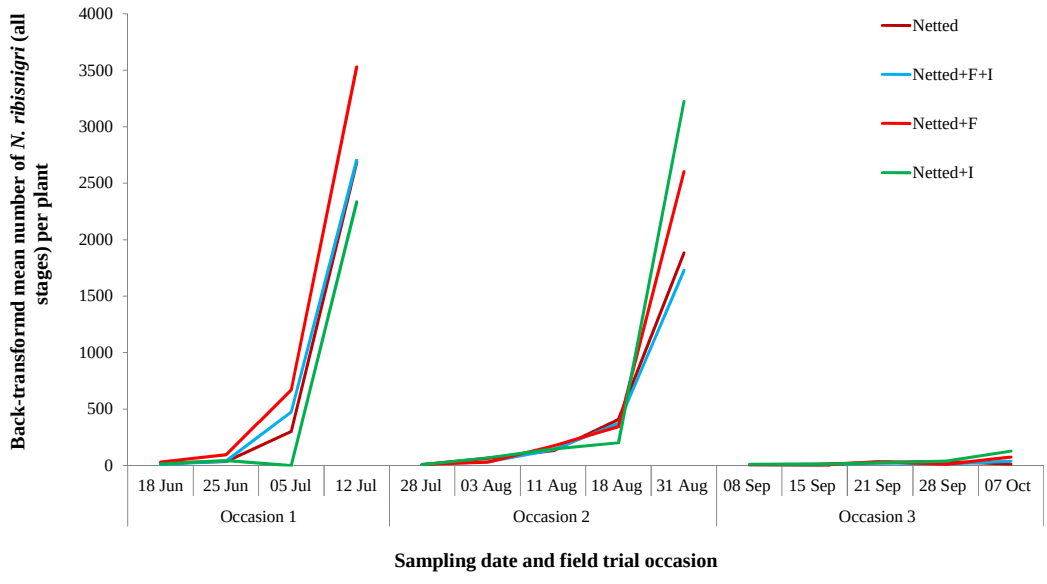
Aphids

Figure 6.1.4a and b shows the back-transformed mean number of *N. ribisnigri* (all stages) recorded per plant for netted treatments and open plots at each sampling date over the three field trial occasions. Netted treatments had significantly higher numbers of *N. ribisnigri* compared with open and control treatments, particularly for Occasions 1 and 2. During Occasion 1 and 2, aphid numbers per plant on the netted treatments were initially low following inoculation and then continued to increase rapidly up to the last sampling date for that occasion. Aphid numbers were higher on the first occasion than the second. During Occasion 3, numbers of *N. ribisnigri* remained low. During sampling a high number of alates *N. ribisnigri* were observed on the underside of the nets.

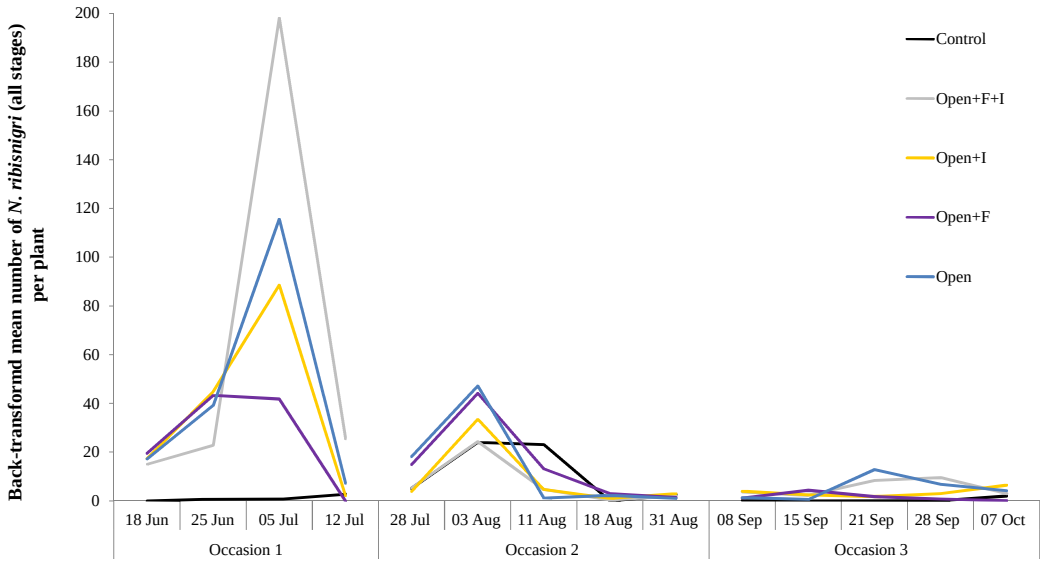
When considering the open plots, aphid numbers during Occasion 1 increased from 18 June until 5 July (Figure 6.1.4b). By 12 July a dramatic decrease in aphid numbers had occurred. Numbers decreased in the Open+F+I treatment from 198.17 aphids per plant to 25.50 per plant, on the Open+I treatment from 88.50 per plant to 2 per plant, on the Open+F treatment from 41.83 per plant to 0 per plant and on the open control plot (non-inoculated) from 115.67 to 7.25 per plant. Unfortunately, data for a fifth sampling week on Occasion 1 plots were not collected as severe rainfall made the plants too wet to sample. Therefore, it is not known whether this decline continued. This population crash was not observed on the netted plots.

A similar, but smaller, decrease was also observed on Occasion 2 where an increase in numbers of *N. ribisnigri* was observed on 28 July and 3 August for the open and control plots, which was then followed by a decline on 11 August, after which aphid numbers remained low for the remainder of the sampling occasions. Aphid numbers on plants in the netted plots peaked later than those in open treatments in Occasions 1 and 2. Aphid numbers also peaked on Occasion 3 in the netted plots, but no clear peak was identified for the open plots.

The back-transformed mean numbers of *N. ribisnigri* on the control plants throughout Occasion 1, 2 and 3 that had not been infested artificially generally remained low, peaking at only 24 aphids per plant on 3 August (Figure 6.1.4b).



a)



b)

Figure 6.1.4a-b Back-transformed mean number of *N. ribisnigri* (all stages) per plant recorded on **a)** netted treatments and **b)** open plots, at each sampling date during three field trials (Occasions 1-3).

The numbers of alate *N. ribisnigri* recorded on each of the netted plots (Figure 6.1.5), show a similar pattern when compared with the mean number of non-alate *N. ribisnigri* (includes apterae and nymphs) in Figure 6.1.6. Both alate and non-alate *N.*

ribisnigri displayed population peaks on 12 July and 31 August for Occasions 1 and 2, but the numbers of alates per plant were much lower than for the numbers of non-alate stages per plant. Whether a further increase in numbers of alate and non-alate *N. ribisnigri* would have been observed for netted treatments following 12 July is again unknown as a fifth sampling date was not possible. Those treatments with the highest number of non-alate aphids per plant did not always result in the highest number of alates per plant.

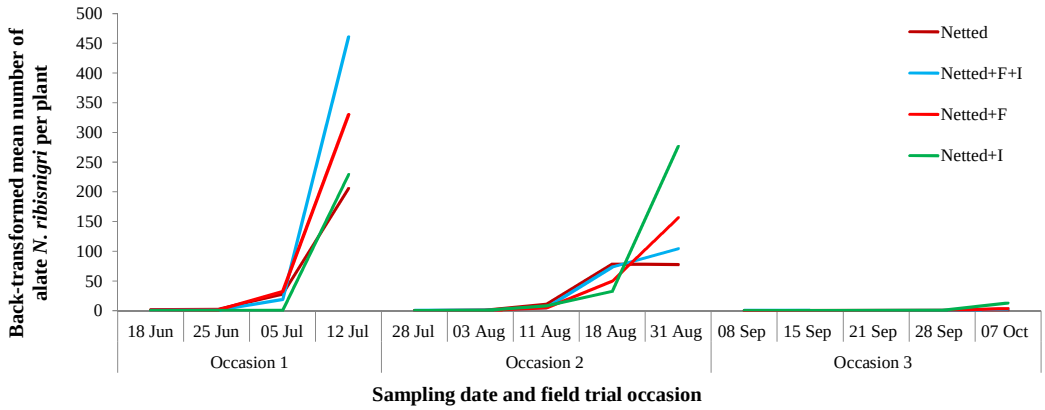


Figure 6.1.5 Back-transformed mean number of alate *N. ribisnigri* per plant for the four netted plots at each sampling date during three field trials.

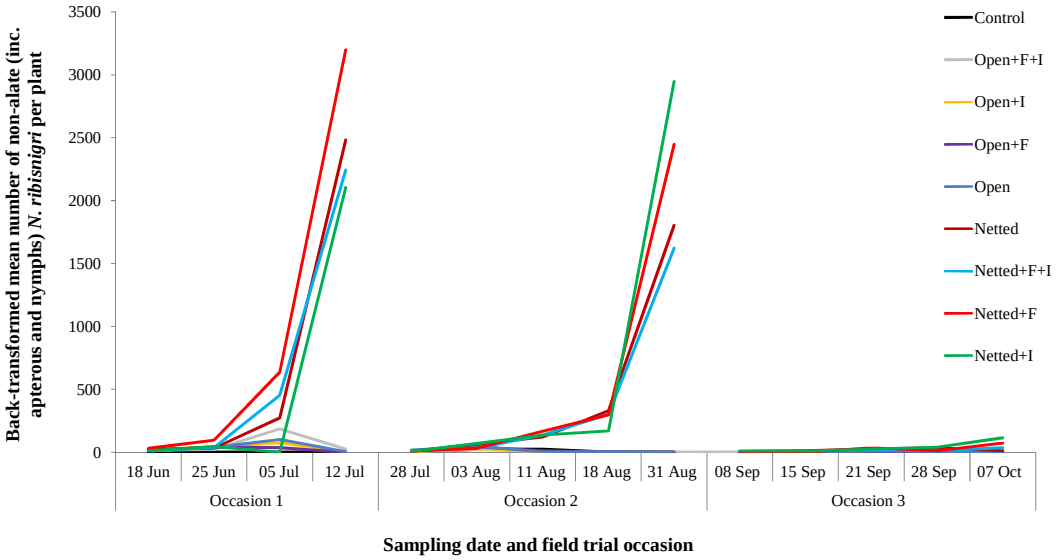
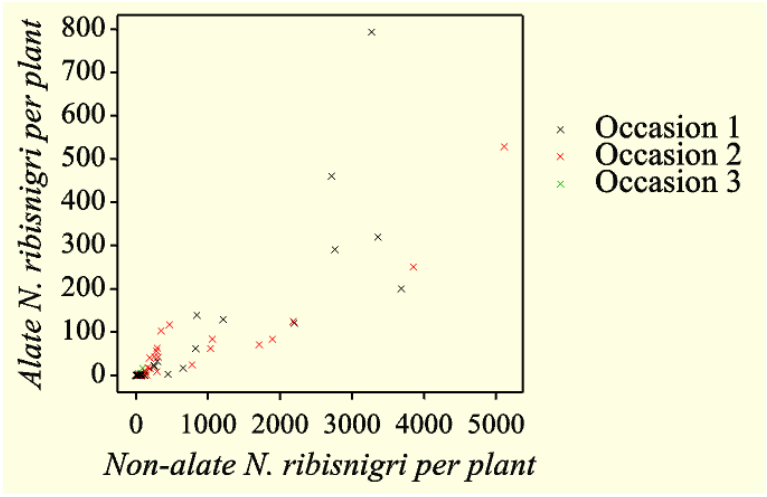


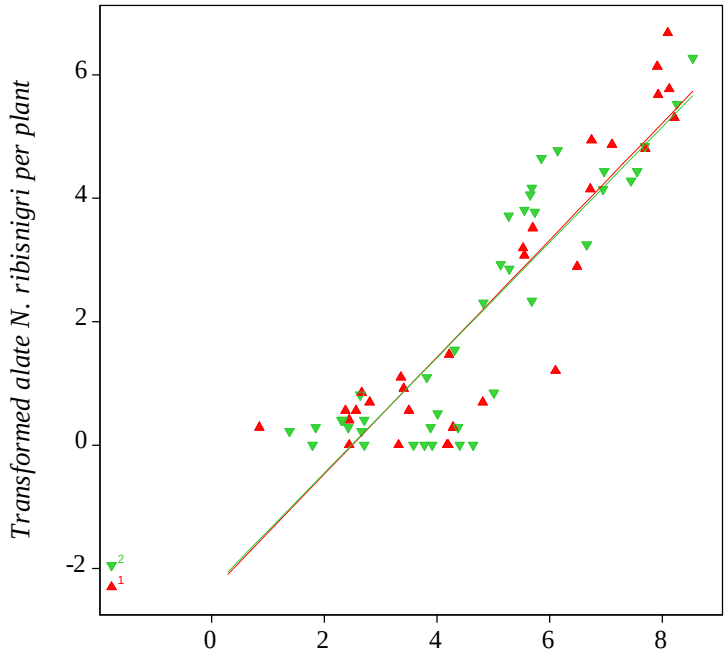
Figure 6.1.6 Back-transformed mean number of non-alate *N. ribisnigri* (including apterae and nymphs) per plant for the nine treatments at each sampling date during three field trials.

Figure 6.1.7a shows the relationship between the numbers of alate and non-alate *N. ribisnigri* for the netted plots for each of the field trial occasions. In the case of Occasion 1 and 2, as the numbers of non-alate forms increased, the numbers of alates also increased. The data for Occasion 3 do not show such a strong relationship as numbers of non-alate and alate aphids remained low.

Performing Pearson R correlations between the numbers of non-alate and alate aphids for each occasion demonstrated a strong and positive correlation coefficient (r) for Occasions 1 (r= 0.83, d.f. 30, p<0.001) and 2 (r= 0.93, d.f. 40, p=0.000) with highly significant probabilities of there being a relationship between them. The coefficient for Occasion 3 was lower, as expected, (r= 0.76, d.f. 40, p<0.001) but still demonstrated a relationship. Figure 6.1.7b shows the data and fitted lines for Occasions 1 and 2 with both X and Y variables transformed using LOG (data+1).



a)



b)

Figure 6.1.7 a-b **a)** Scatter graph of untransformed counts of alate and non-alate *N. ribisnigri* for each of the three field trial occasions for the netted plots **b)** Linear regression of the number of alates and non-alate *N. ribisnigri* (LOG(data+1) for Occasions 1 (Y= -2.367+0.9482*x*) and 2 (Y= -2.324+0.9351*x*) for the netted plots.

Figure 6.1.8 shows the mean percentage of the total population per plant which were alates at each sampling date during the three field trial occasions in the netted treatments. Generally, during Occasion 1 and 2 the percentage of alates increased at

each sampling date, except on 31 August 2010 (Occasion 2) where the percentage of alates decreased by 16%. During Occasion 3 no clear trend was observed and the percentage of alates fluctuated. The percentage of alates in the total population never exceeded 20%.

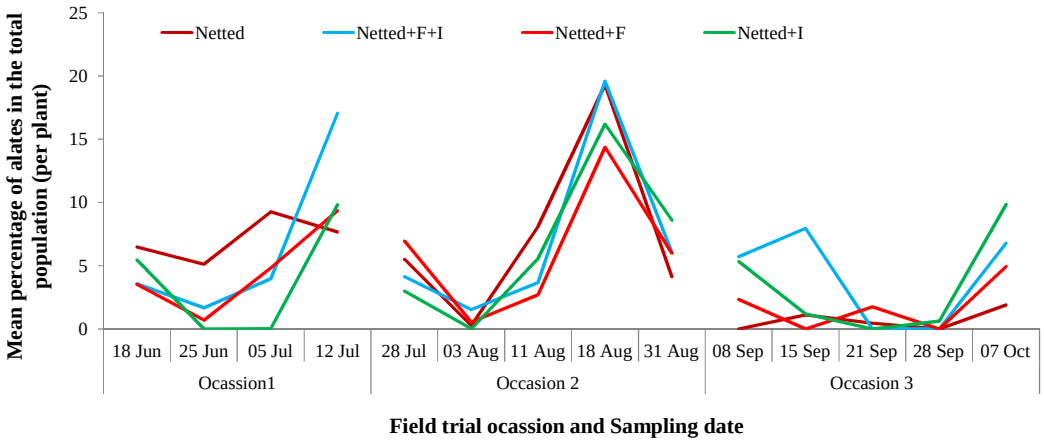


Figure 6.1.8 Mean percentage of the total population per plant which were alates at each sampling date during three field trials for the netted treatments.

With the open plots, similar patterns were observed when comparing non-alate and alate *N. ribisnigri* (Figure 6.1.6 and 6.1.9). Unlike the netted plots, peak numbers occurred on 5 July and a decline was observed by 12 July.

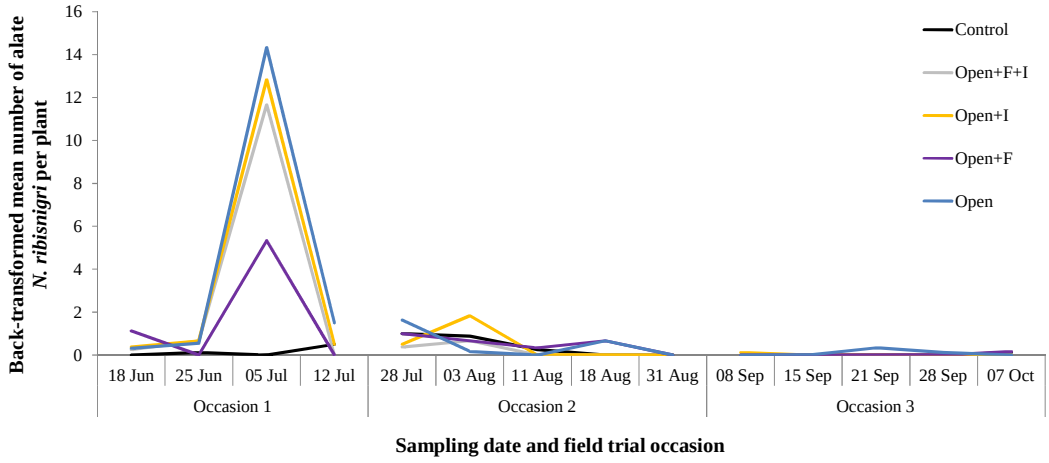
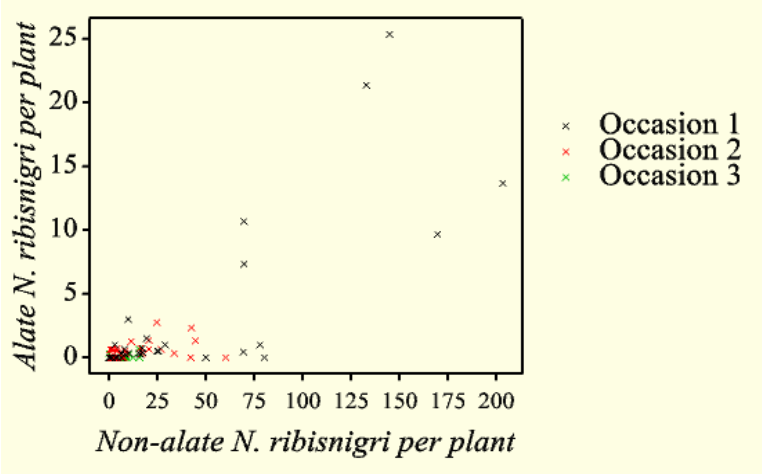


Figure 6.1.9 Back-transformed mean number of alate *N. ribisnigri* per plant for the four open plots at each sampling date during the three field trials.

Figure 6.1.10a shows the relationship between alate and non-alate *N. ribisnigri* for the open plots for each of the field trials. This is similar to the netted plots, but not as strong. When Pearson R correlations were performed, a high positive correlation was observed between the number of alate and non-alate *N. ribisnigri* for Occasion 1 (r= 0.79, d.f. 32, p<0.001) but the relationship was weaker for Occasions 2 (r= 0.44, d.f. 40, p=0.004) and 3 (r= 0.40, d.f. 40, p=0.01). Figure 6.1.10b shows the fitted lines for Occasions 1 and 2 with both X and Y variables transformed using (LOG(data+1).

a)



b)

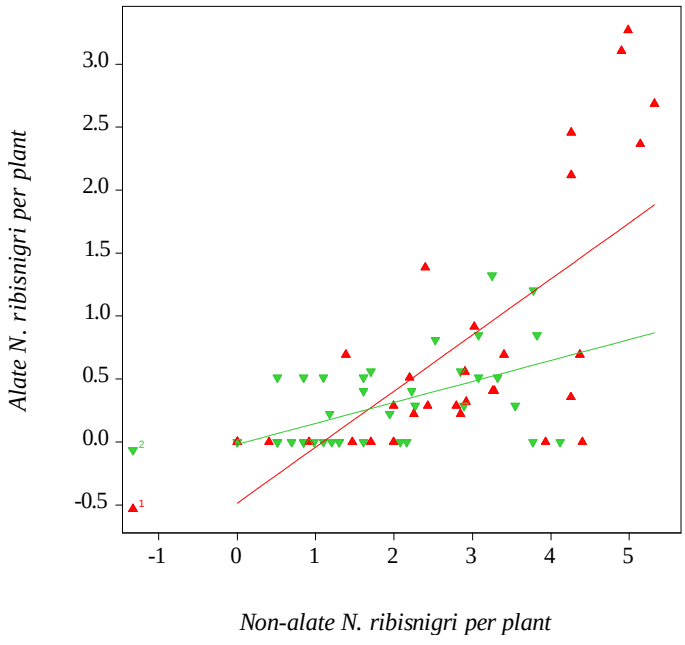


Figure 6.1.10 a-b **a)** Scatter graph of untransformed counts of alate and non-alate *N. ribisnigri* for each of the three field trial occasions for the open plots **b)** Linear regression of the number of alates versus non-alate *N. ribisnigri* for Occasions 1 (Y= -0.486+0.4453*x)* and 2 (Y= -0.018+0.1665*x*) for the open plots.

Figure 6.1.11 shows the mean percentage of the total population per plant which were alates at each sampling date during the three field trial occasions in the open plots. During Occasion 1 the percentage of alates in all treatments increased until 5 July 2010. Following this the percentage alates in two treatments (Open+I and Open) continued to increase while the other treatments decreased (Open+F+I and Open+F). During Occasion 2, two treatments (Open and Open+F) increased to a peak on 18

August 2010, while few alates were observed in the other treatments. During Occasion 3, the percentage of alates only exceeded 3% on 7 October 2010 when 100% alates were observed. This was because only one aphid (an alate) was found.

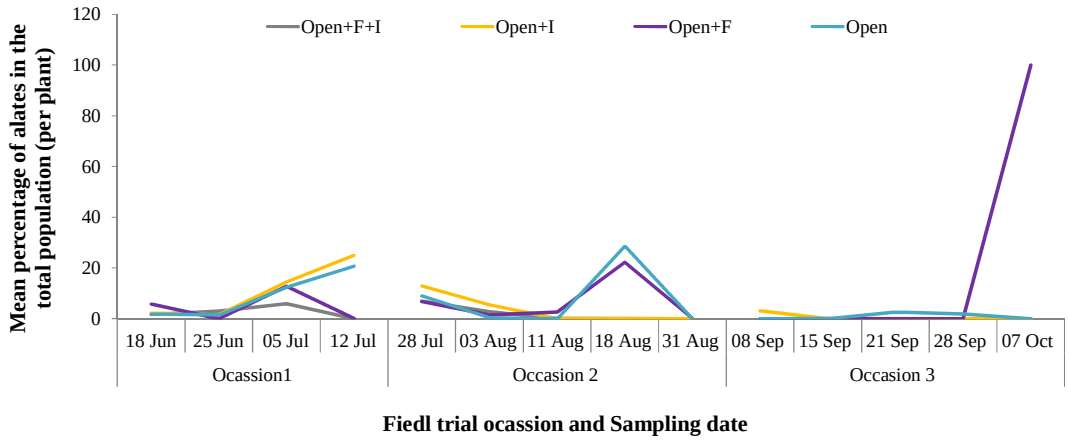
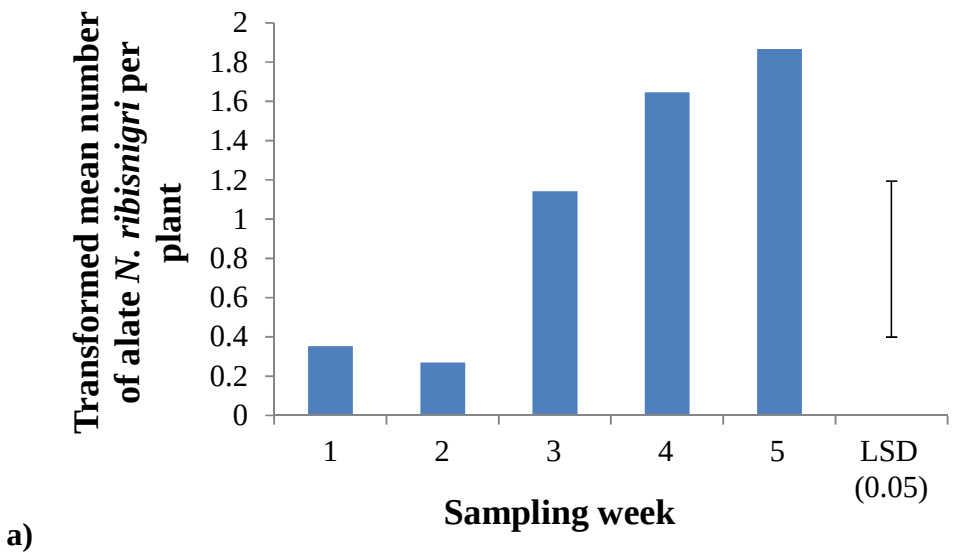
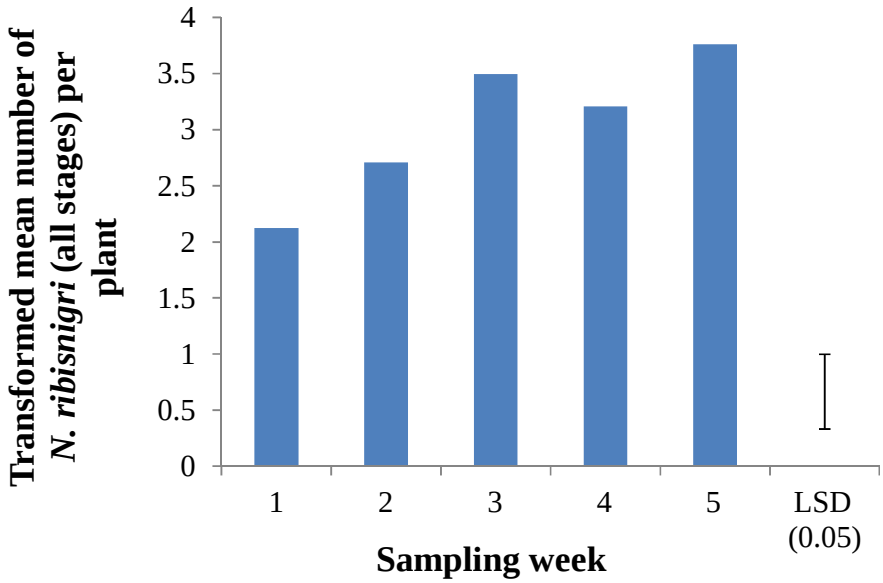


Figure 6.1.11 Mean percentage of the total population per plant which were alates at each sampling date during three field trials in the open plots.

An ANOVA was performed on the numbers of alate and all stages of *N. ribisnigri* recorded. A significant effect of sampling week was observed for both alate ($F(4,18)= 7.40$, $p=0.001$) and all stages of *N. ribisnigri* per plant ($F(4,18)= 8.77$, $p<0.001$). Figure 6.1.12a and b shows the effect of sampling week on the numbers of alates and all stages of *N. ribisnigri* respectively. Later sampling weeks had significantly more aphids than Sampling Week 1.



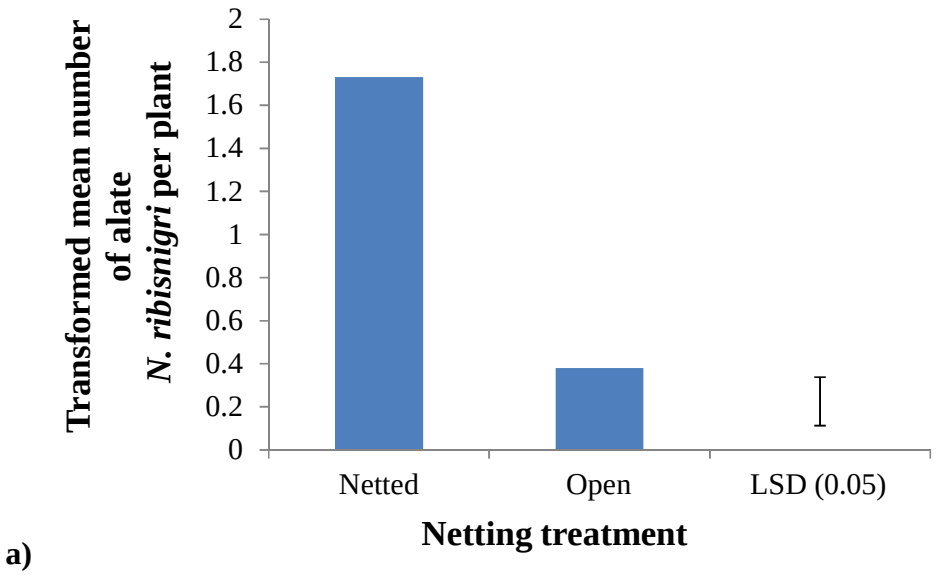
a)



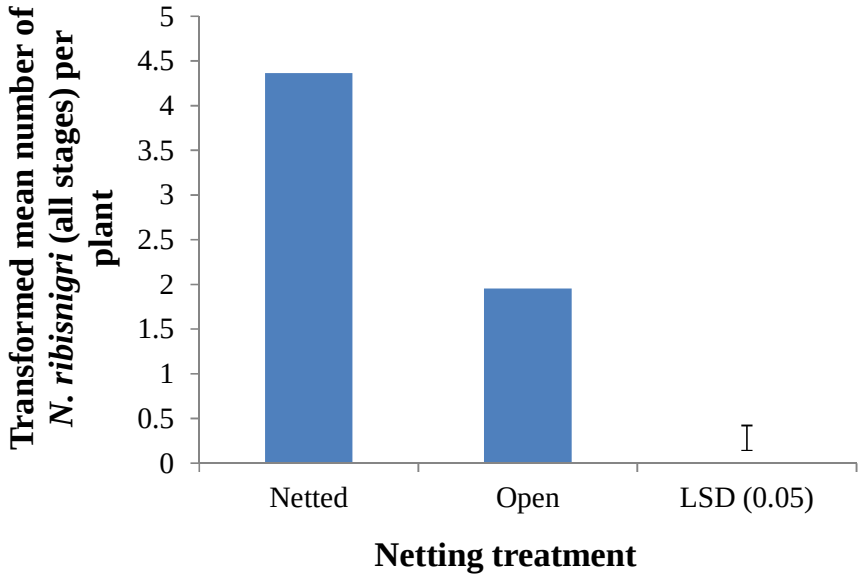
b)

Figure 6.1.12 a-b Transformed mean number of **a)** alates **b)** all stages of *N. ribisnigri* per plant from the ANOVA for the effect of sampling week.

A significant effect of netting treatment was observed for both alate ($F(1,159)= 140.98$, $p<0.001$) and all stages of *N. ribisnigri* ($F(1,159)= 286.77$, $p<0.001$). Figure 6.1.13a and b shows the effect of netting treatment on the numbers of alate and all stages of *N. ribisnigri* respectively where netted plots always have higher numbers of aphids than open plots.



a)



b)

Figure 6.1.13 a-b Transformed mean number of **a)** alate **b)** all stages of *N. ribisnigri* per plant from the ANOVA for the effect of netting treatment.

An interaction was observed between netting treatment and sampling week for both alate ($F(4,159)= 34.90$, $p<0.001$) and all stages of *N. ribisnigri* ($F(4,159)= 43.02$, $p<0.001$). When considering the interactions between sampling week and netting treatment, significantly more alate (Figure 6.1.14) and all stages of *N. ribisnigri* (Figure 6.1.15) were recorded per plant on netted plots than on open plots, except for Sampling Weeks 1 and 2. From Sampling Week 3 onwards, the difference between

netted and open plots increased each week, with the numbers of aphids increasing on plants in netted plots but not in open plots. No effects of fungicide or insecticide application were observed.

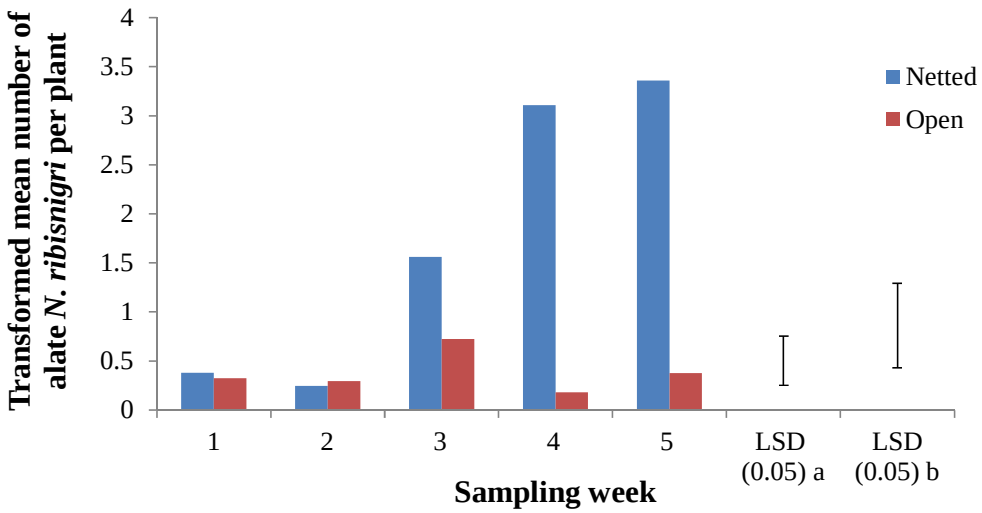


Figure 6.1.14 Transformed mean number of alate *N. ribisnigri* per plant from the ANOVA for the interaction between sampling week and netting treatment. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

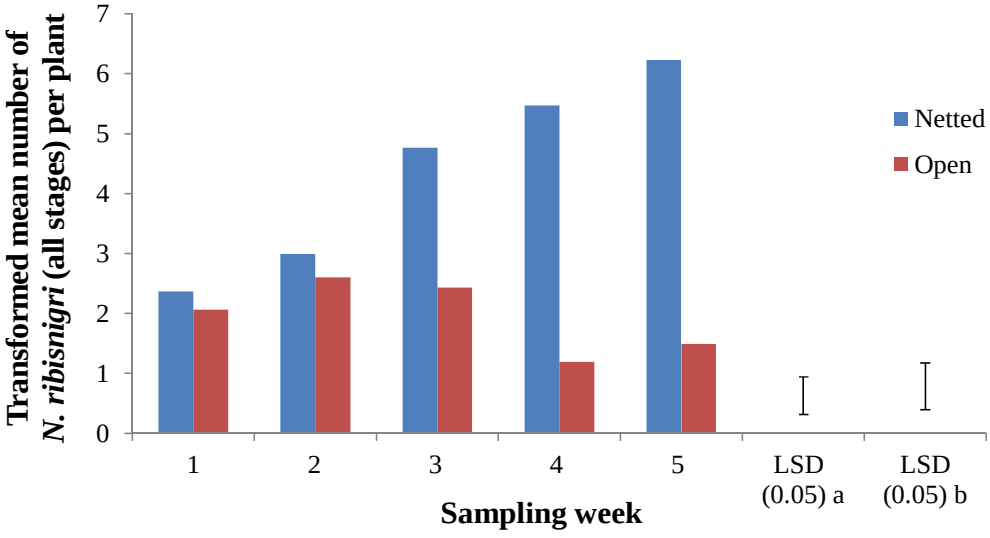


Figure 6.1.15 Transformed mean number of all stages of *N. ribisnigri* per plant from the ANOVA for the interaction between sampling week and netting treatment. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

An ANOVA was also performed on the number of natural enemies recorded to determine whether there was an effect of netting or fungicide or insecticide treatment.

Parasitized aphids

There was a significant effect of netting treatment ($F(1,159)= 8.50$, $p=0.004$) on the mean number of parasitized aphids per plant. As shown in Table 6.1.16, significantly more parasitized aphids per plant were found in open plots.

Table 6.1.16 Transformed mean number of parasitized aphids per plant from the ANOVA for the effect of netting treatment.

Treatment	**Mean**
Netted	0.247
Open	0.432
LSD	0.1259
d.f.	159

There was an interaction between netting treatment and sampling week on the number of parasitized aphids per plant ($F(4,159)= 9.67$, $p<0.001$). Figure 6.1.17 shows the interaction, where significantly more parasitized aphids were found on open plots compared with netted plots in Sampling Weeks 1-4. In Sampling Weeks 4 and 5, the number of parasitized aphids in netted treatments increased, indicating that they were present in treatments where they were supposed to be excluded. In Sampling Week 5 more parasitized aphids per plant were observed in netted plots.

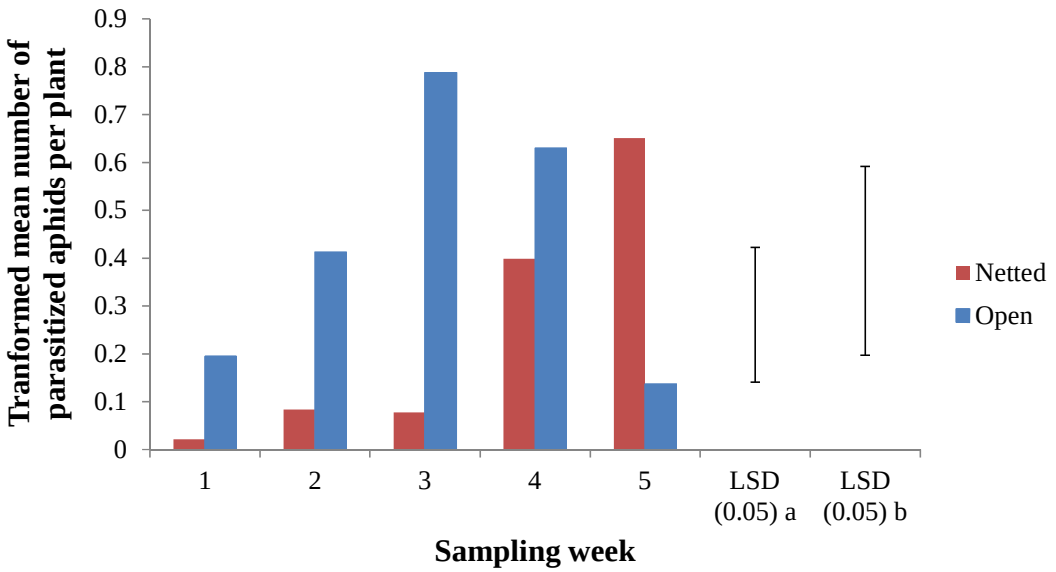


Figure 6.1.17 Transformed mean number of parasitized aphids per plant from the ANOVA for the interaction between sampling week and netting treatment. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

The ANOVA indicated that there was a significant interaction between fungicide and insecticide on the number of parasitized aphids per plant ($F(1,159)= 6.09$, $p=0.015$). Figure 6.1.18 illustrates the means and LSD of this output which does not support this significant effect. This anomaly can be described as a cross-over interaction where there were no individual effects of insecticide and fungicide but a major interaction between the two treatments. This suggest that not applying both insecticide and fungicide, or applying both, reduced the number of parasitized aphids present, while applying them individually was beneficial and increased the number of parasitized aphids.

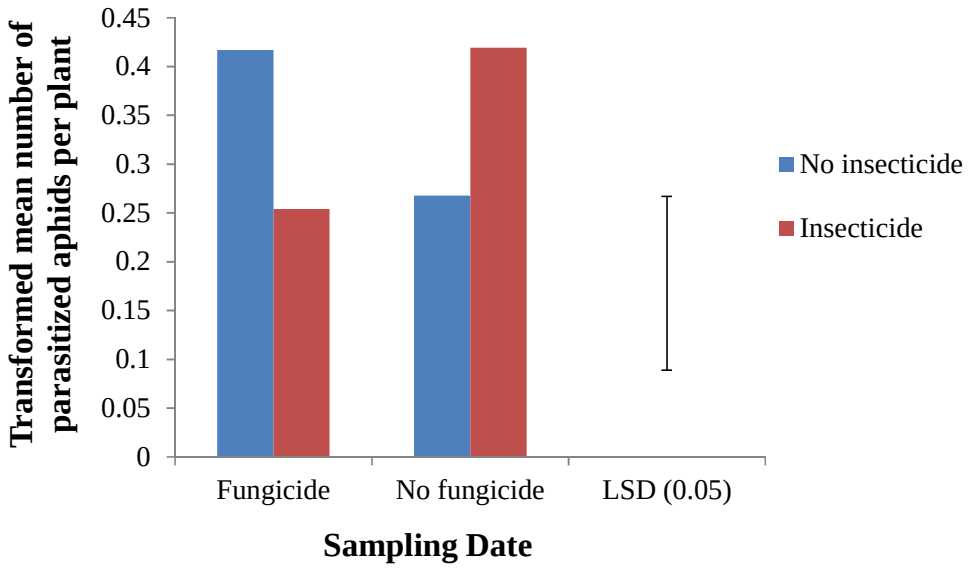


Figure 6.1.18 Transformed mean number of parasitized aphids per plant from the ANOVA for the interaction between fungicide and insecticide.

An interaction between netting, fungicide and insecticide treatments on the number of parasitized aphids per plant was observed ($F(1,159)= 9.45$, $p=0.002$). As shown in Figure 6.1.19, netted plots with neither or both fungicide and insecticide applications had significantly less parasitized aphids than those treated with either fungicide or insecticide. The numbers of parasitized aphids in open plots were similar regardless of the insecticide or fungicide treatment.

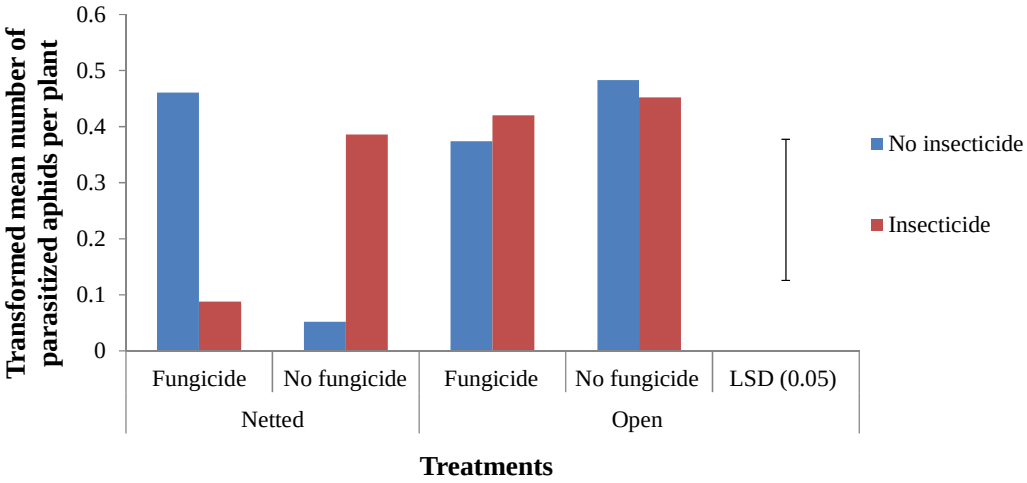


Figure 6.1.19 Transformed mean number of parasitized aphids per plant from the ANOVA for the interaction between netting, fungicide and insecticide treatments.

Syrphid larvae

A significant effect of netting treatment on the number of syrphid larvae per plant ($F(1,159)= 56.88$, $p<0.001$) was observed. As shown in Table 6.1.20, significantly more syrphid larvae per plant were observed in open plots.

Table 6.1.20 Transformed mean number of syrphid larvae per plant from the ANOVA for the effect of netting treatment.

Treatment	Mean
Netted	0.027
Open	0.315
LSD	0.0755
d.f.	159

An interaction between netting treatment and sampling week was also observed ($F(4,159)= 7.34$, $p=0.001$). As shown in Figure 6.1.21, significantly more syrphid larvae were observed per plant in open plots in Sampling Weeks 3-5, while significantly fewer syrphid larvae were present in Sampling Weeks 1 and 2 than in Sampling Weeks 3-5.

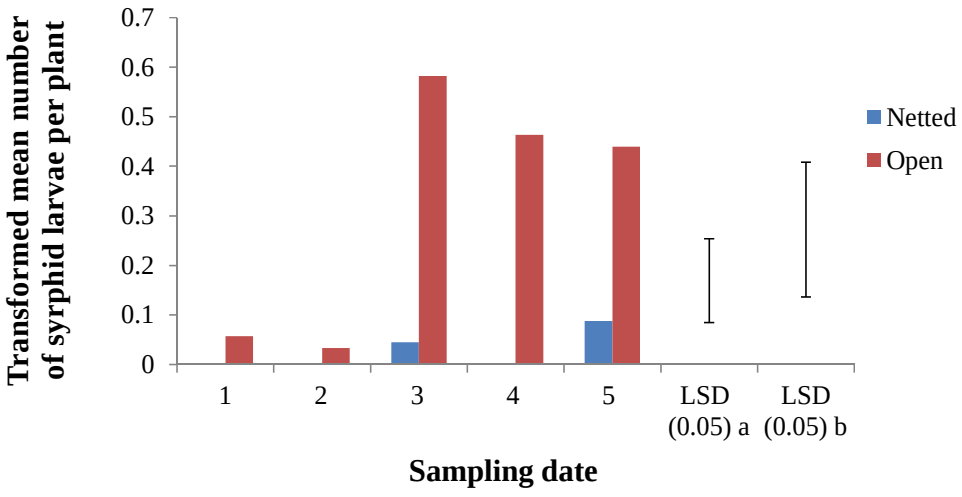


Figure 6.1.21 Transformed mean number of syrphid larvae per plant from the ANOVA for the interaction between sampling week and netting treatment. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

Anthocoridae

A significant effect of netting (Table 6.1.22) was observed, with more Anthocoridae found per plant in open plots ($F(1,159)= 35.31$, $p<.001$).

Table 6.1.22 Transformed mean number of Anthocoridae per plant from the ANOVA for the effect of netting treatment.

Treatment	**Mean**
Netted	0.002
Open	0.157
LSD	0.0515
d.f.	159

An interaction was observed between netting treatment and sampling week ($F(4,159)= 4.52$, $p=0.002$). As shown in Figure 6.1.23, more Anthocoridae were observed on open plots, particularly during Sampling Weeks 3-5. For open plots, significantly less Anthocoridae were present in Sampling Weeks 1 and 2 compared with Sampling Weeks 3-5. Negative estimated mean values were observed for this interaction, indicating there were too many zero values in the data set and the ANOVA analysis could not provide good estimates for these zero values based on the data available.

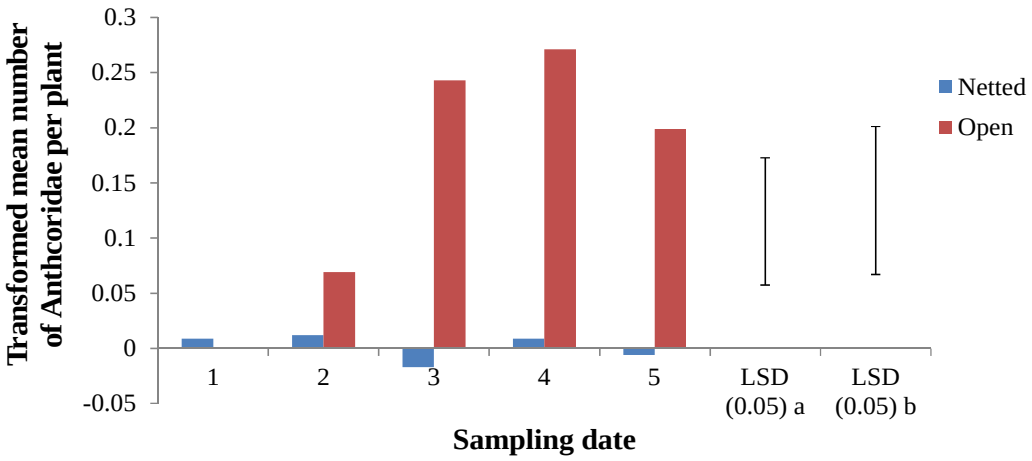


Figure 6.1.23 Transformed mean number of Anthocoridae per plant from the ANOVA for the interaction between sampling week and netting. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

A significant effect of fungicide on the numbers of Anthocoridae was observed ($F(1,159)= 4.96$, $p=0.027$); more were present on plants from fungicide treated plots (Table 6.1.24).

Table 6.1.24 Transformed mean number of Anthocoridae per plant from the ANOVA for the effect of fungicide.

Treatment	Mean
Fungicide	0.108
No fungicide	0.050
LSD	0.0515
d.f.	159

As shown in Figure 6.1.25, there was an interaction between fungicide and insecticide ($F(1,159)= 4.36$, $p=0.038$). There were fewer Anthocoridae per plant from plots which were treated with insecticide only, or from untreated plots, than from plots treated with both insecticide and fungicide treatments.

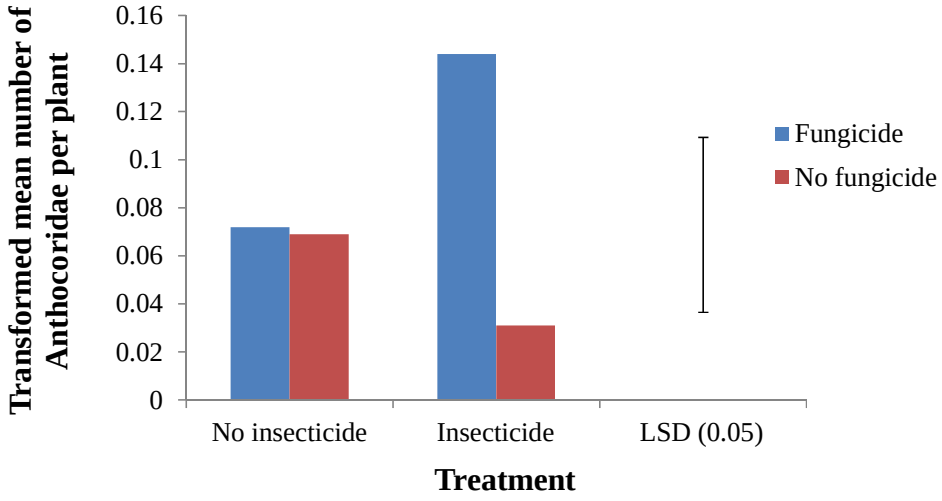


Figure 6.1.25 Transformed mean number of Anthocoridae per plant from the ANOVA for the interaction of insecticide and fungicide.

Neuroptera

The numbers of Neuroptera were very low during the trial, resulting in negative values for some of the means, indicating a lack of data. Therefore, the ANOVA output should be interpreted with caution. There was a significant effect of netting ($F(1,159)= 12.52$, $p<0.001$) with more Neuroptera in open plots (Table 6.1.26).

Table 6.1.26 Transformed mean number of Neuroptera per plant from the ANOVA for the effect of netting treatment.

Treatment	Mean
Netted	-0.0011
Open	0.0351
LSD	0.2020
d.f.	159

An interaction was observed between netting treatment and sampling week ($F(4,159)= 4.72$, $p=0.001$). Neuroptera were only present in Sampling Weeks 4 and 5 on plants from open plots and this was significantly different to the other sampling

weeks (Figure 6.1.27). Again, negative estimates of mean values indicate a lack of data.

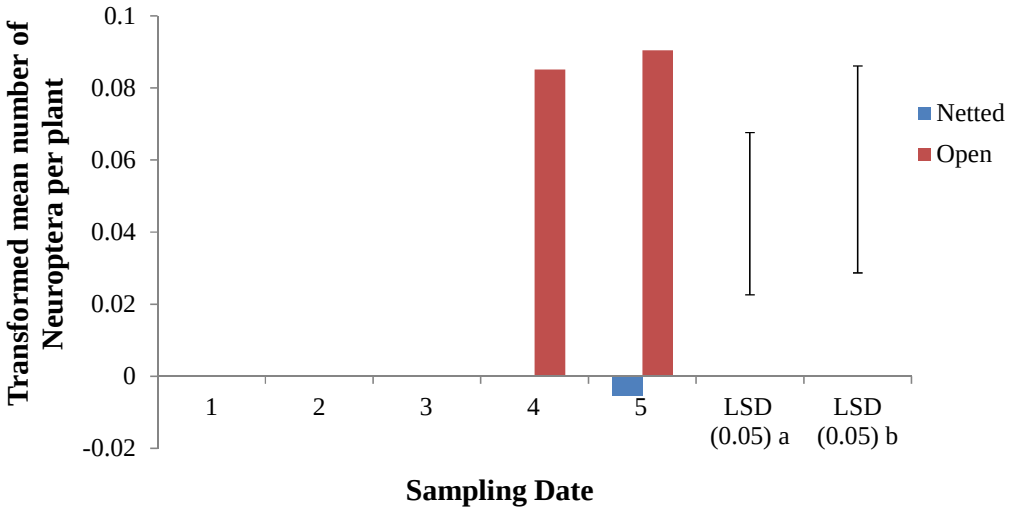


Figure 6.1.27 Transformed mean number of Neuroptera per plant from the ANOVA for the interaction between sampling week and netting treatment. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

A significant effect of the interaction between sampling week, fungicide and insecticide ($F(4,159)= 3.51$, $p=0.009$) and also of sampling week, netting, fungicide and insecticide ($F(4,159)= 3.51$, $p=0.009$) were observed, but they were not used due to a lack of data.

Araneae

There was a significant effect of sampling week ($F(4,18)= 10.42$, $p<0.001$) on the numbers of spiders. Significantly more spiders were found in Sampling Weeks 3-5 (Table 6.1.28).

Table 6.1.28 Transformed mean number of Araneae per plant from the ANOVA for the effect of sampling week.

Sampling Week	**Mean**
1	0.060
2	0.089
3	0.347
4	0.425
5	0.473
LSD	0.1770
d.f.	18

There was a significant effect of netting treatment ($F(1,159)= 41.68$, $p<0.001$) and more spiders were found in plants from open plots (Table 6.1.29).

Table 6.1.29 Transformed mean number of Araneae per plant from the ANOVA for the effect of netting treatment.

Treatment	**Mean**
Netted	0.159
Open	0.398
LSD	0.0730
d.f.	159

There was an interaction between sampling week and netting treatment ($F(4,159)= 4.42$, $p=0.002$). With the exception of Sampling Week 1, significantly more spiders were found on plants from open plots (Figure 6.1.30), and the difference increased from Sampling Week 2-4. There were more spiders on plants from open plots in

Sampling Weeks 3, 4 and 5 compared with Sampling Week 1. For plants from netted plots, there were more spiders in Sampling Week 5 than in Sampling Week 1.

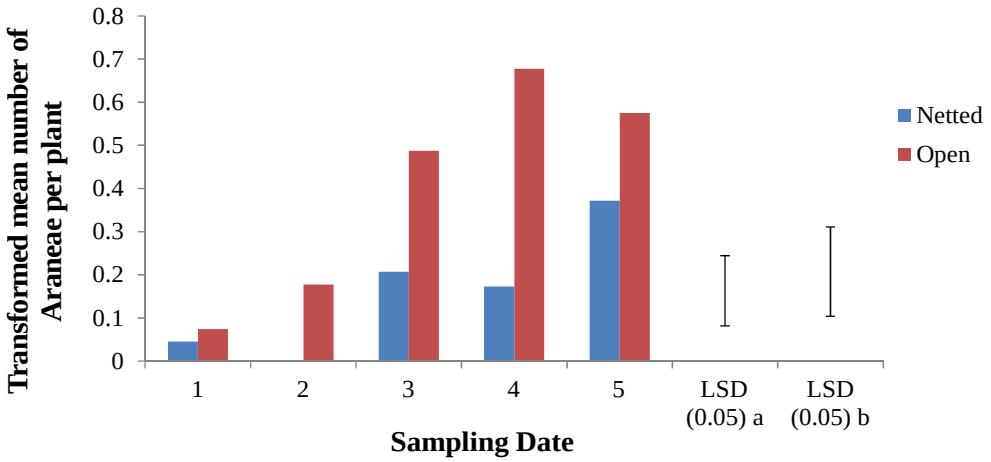


Figure 6.1.30 Transformed mean number of Araneae per plant from the ANOVA for the interaction between sampling week and netting. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

Total number of natural enemies

When the numbers of Anthocoridae, Coccinellidae, Neuroptera, parasitized aphids and syrphid larvae were summed, there was an overall effect of sampling date ($F(4,18)= 4.94$, $p=0.007$). Table 6.1.31 shows that there were significantly more natural enemies in Sampling Weeks 3-5 compared with Sampling Week 1.

Table 6.1.31 Transformed mean number of total natural enemies per plant from the ANOVA for the effect of sampling week.

Sampling Week	Mean
1	0.130
2	0.280
3	0.649
4	0.779
5	0.701
LSD	0.3814
d.f.	18

When considering the effect of netting treatment, significantly more natural enemies were observed per plant from open plots ($F(1,159)= 49.86$, $p<0.001$) (Table 6.1.32).

Table 6.1.32 Transformed mean total number of natural enemies per plant from the ANOVA for the effect of netting treatment.

Treatment	Mean
Netted	0.268
Open	0.748
LSD	0.1342
d.f.	159

There was an interaction between sampling week and netting treatment ($F(4,159)= 8.31$, $p<0.001$). There were significantly more natural enemies in Sampling Weeks 2, 3 and 4 in plants from open plots (Figure 6.1.33). Between sampling weeks, plants from open plots contained significantly more natural enemies in Sampling Weeks 3, 4 and 5 than in Sampling Week 1. Significantly more natural enemies were found in plants from netted plots in Sampling Week 5 than in Sampling Week 1.

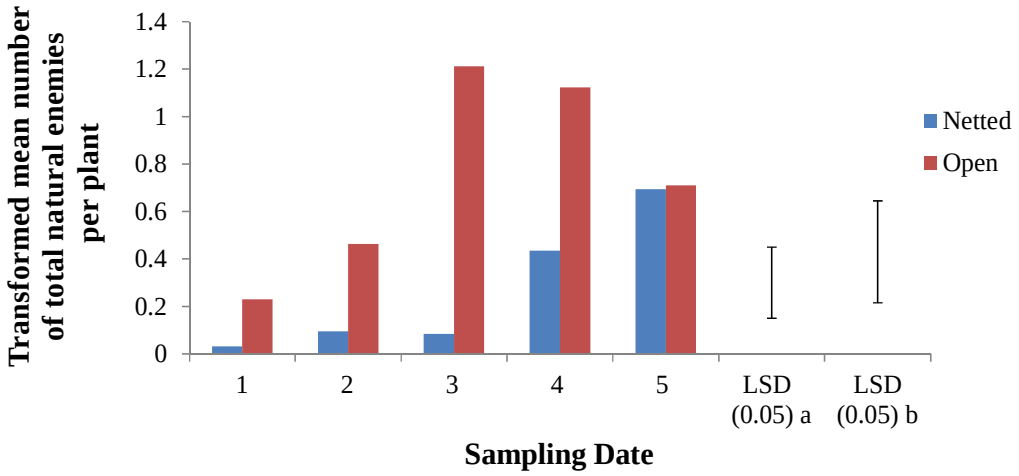


Figure 6.1.33 Transformed mean total number of natural enemies per plant from the ANOVA for the interaction between sampling week and netting. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

Figure 6.1.34 shows the slight interaction between insecticide and fungicide treatments ($F(1,159)= 4.01$, $p=0.047$) where the number of natural enemies found in plants taken from plots with no application of fungicide and insecticide were significantly different to those taken from plots where only fungicide was applied.

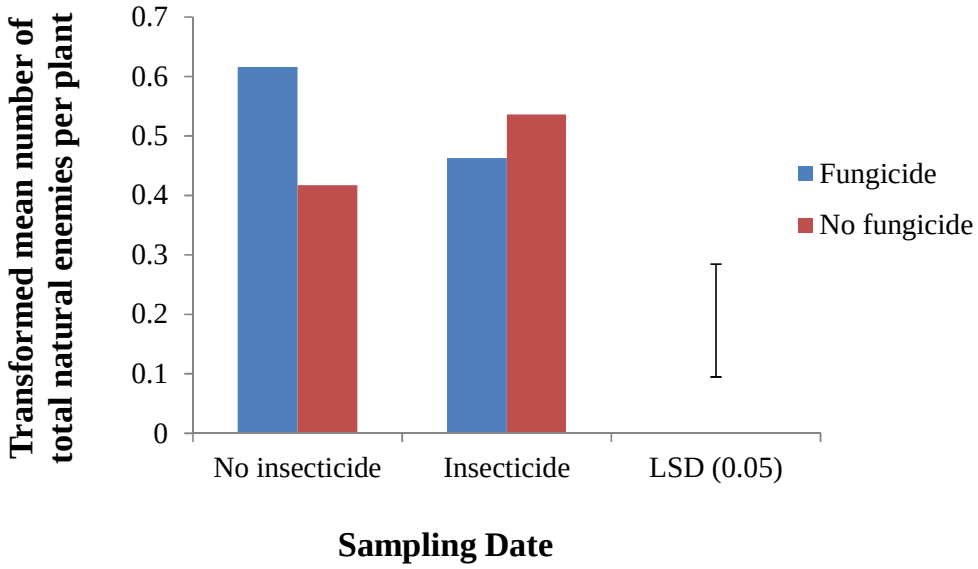


Figure 6.1.34 Transformed mean number of natural enemies per plant from the ANOVA analysis for the interaction of insecticide and fungicide.

Finally, an interaction between netting treatment, insecticide and fungicide ($F(1,159)= 9.42$, $p=0.003$) was observed which again confirms that numbers of natural enemies were generally higher in open plots (Table 6.1.35). When compared with one another, plants from open treatments had similar numbers of predators regardless of the application of, or absence of, fungicide and/or insecticide. However, netted plots had a lower number of natural enemies when fungicide and insecticide were combined or when they were both absent.

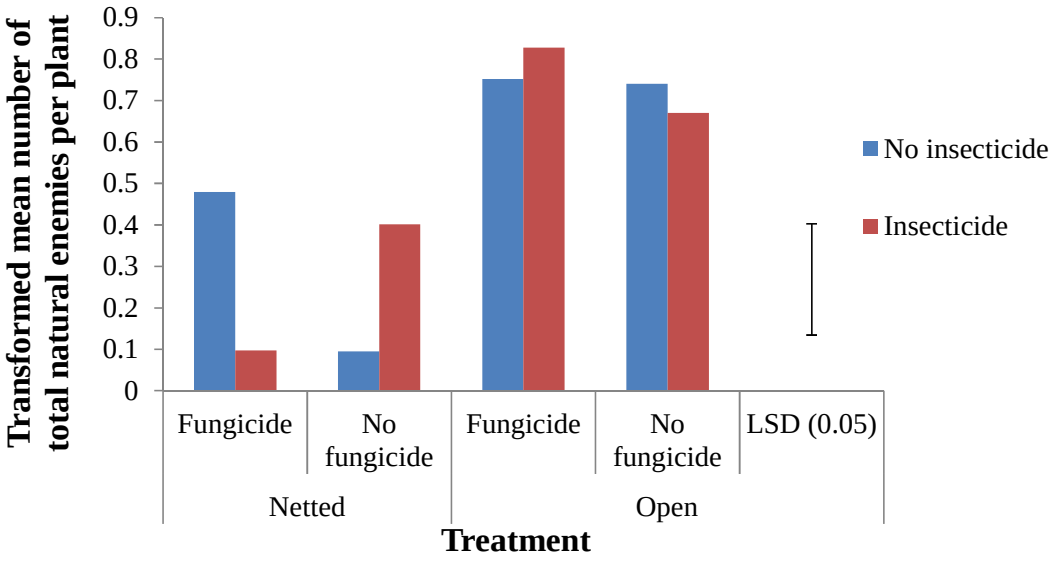


Figure 6.1.35 Transformed mean number of natural enemies from the ANOVA for the interaction of netting, insecticide and fungicide.

When analysing the data on the presence of natural enemies, the main focus was their presence around 5 and 12 July, where a decline in aphid numbers had been identified in open plots. When plotting the back-transformed numbers of *N. ribisnigri* (all stages) per plant and the numbers of Anthocoridae, Neuroptera, parasitized aphids, Araneae and syrphid larvae per plant, only a relationship between parasitized aphids and *N. ribisnigri* (all stages) per plant was observed on 5 July (Figure 6.1.36). The relationship shows that the number of parasitized aphids per plant increased with the number of *N. ribisnigri* (all stages) per plant. However, two extreme responses can be observed in this relationship. One occurs in one of the replicate plots for treatment Open+I, where extremely high numbers of parasitized aphids compared with other treatments occurred, when *N. ribisnigri* numbers were also high. The other anomaly occurs in one of the replicate plots for treatment Open+F+I where no parasitized aphids were observed when *N. ribisnigri* numbers were high.

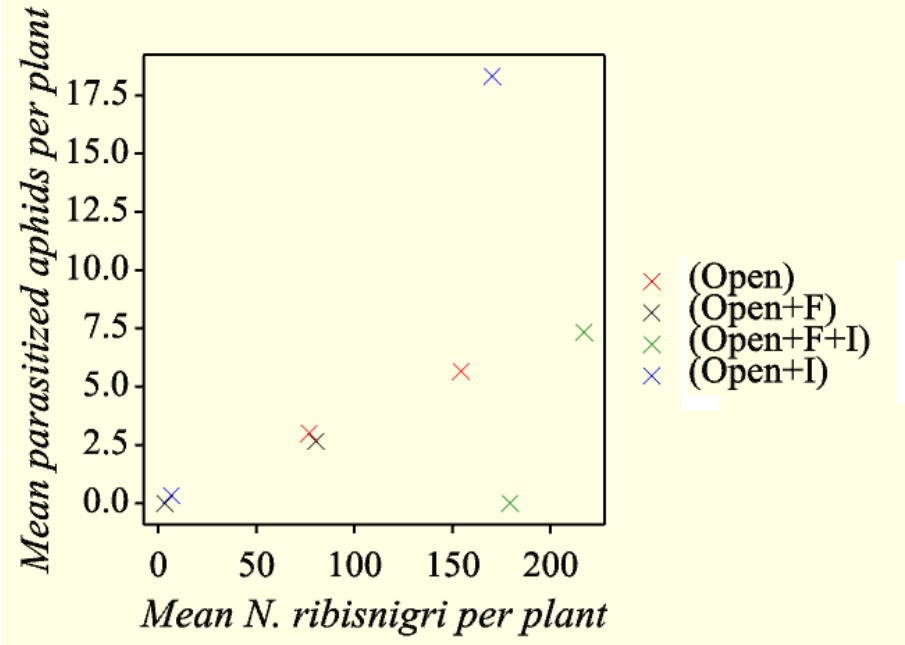


Figure 6.1.36 Back-transformed mean number of *N. ribisnigri* (all stages) per plant and mean number of parasitized aphids per plant on 5 July 2010 sampling date.

As each of these observations is based on the destructive sampling of approximately four plants, these anomalies could be explained by parasitoids parasitizing or not parasitizing aphids more on these lettuce plants by chance, compared to other lettuce plants in other treatment plots. When removing these anomalies and performing a linear regression, it indicated a relationship of 1 parasitized aphid per 30 *N. ribisnigri* (Figure 6.1.37). A strong and positive correlation coefficient was calculated (r=0.10, observations 6, p<0.001).

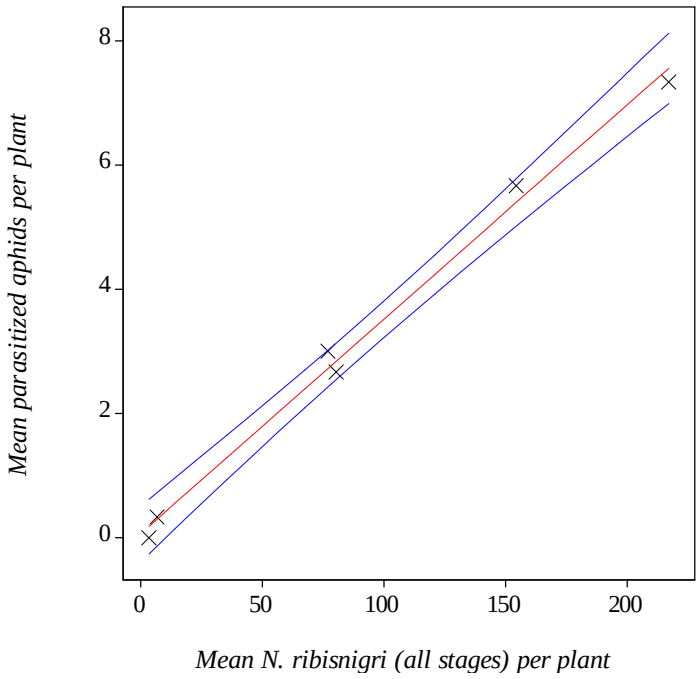


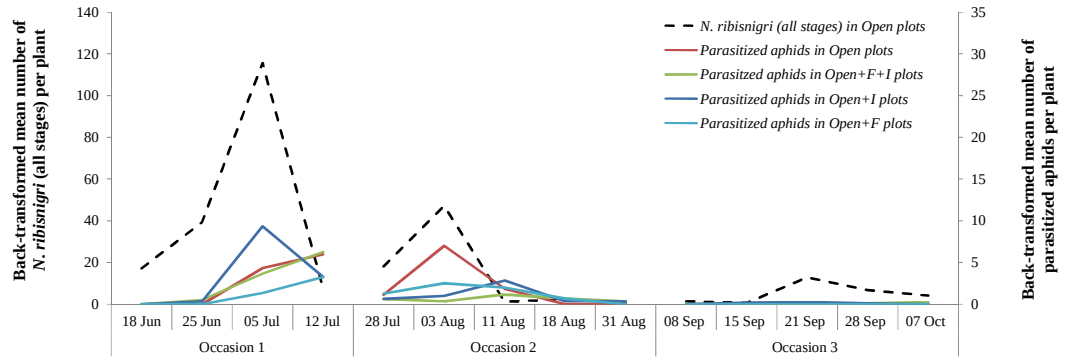
Figure 6.1.37 Linear regression of the back-transformed mean number of *N. ribisnigri* and parasitized aphids per plant on 5 July 2010 sampling date (Y= 0.068+0.03452*x*).

When looking at the number of natural enemies recorded at each sampling date, parasitic wasps (evaluated through the number of parasitized lettuce aphids) and syrphid larvae were the most prevalent natural enemies in the open plots. The numbers of parasitized aphids were higher than the numbers of syrphid larvae, but both peaked on 5 July when the numbers of *N. ribisnigri* also peaked (Figure 6.1.38a and b). On 12 July, however, aphid numbers had declined considerably whilst the parasitized aphids and syrphid larvae were still present but in lower numbers than those seen on 5 July. Whether these parasitized aphids were *N. ribisnigri* is unknown.

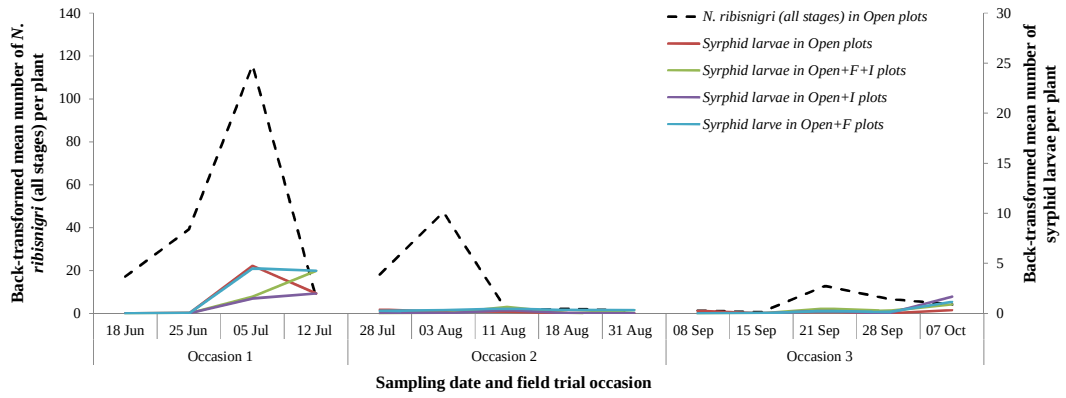
Other predators associated with aphids, such as the Anthocoridae, were recorded in lower numbers, as illustrated in Figure 6.1.38c, where *Anthocoris nemorum* was the most prevalent species. The Hemerobiidae and Chrysopidae (Neuroptera) were also present in low numbers as shown in Figure 6.1.38d; the majority of these were identified as *Micromus variegatus* (Chrysopidae).

When considering the activity of generalist predators such as the Araneae, they were present throughout the three field trials, but displayed one of their two population

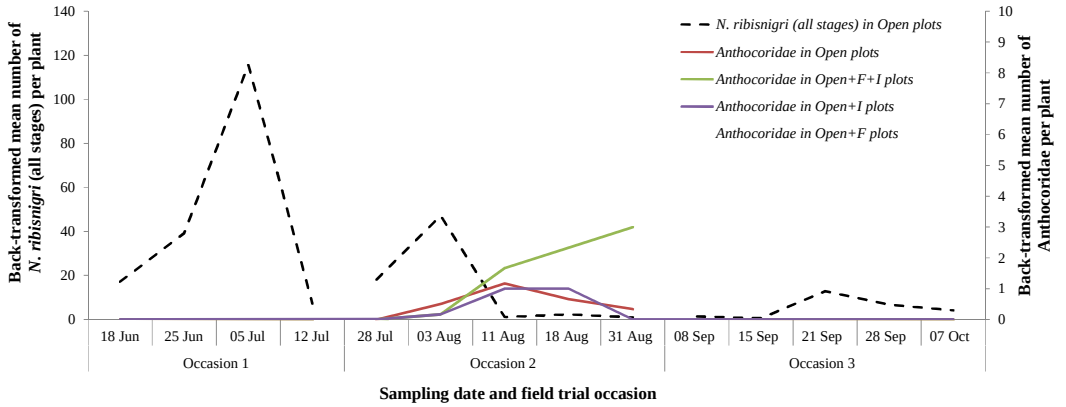
peaks on 12 July, as the number of all stages of *N. ribisnigri* decreased (Figure 6.1.38e). Throughout the trial, some natural enemies were rarely observed, particularly mobile natural enemies such as the Coccinellidae, Hemerobiidae and Chrysopidae.



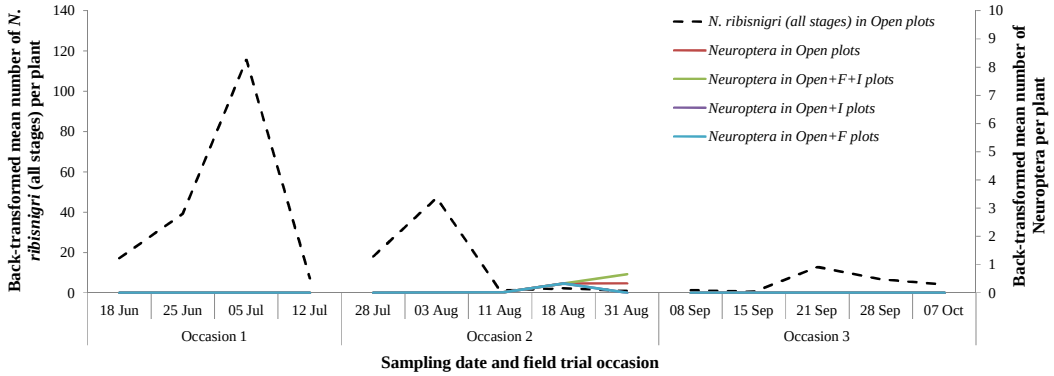
a)



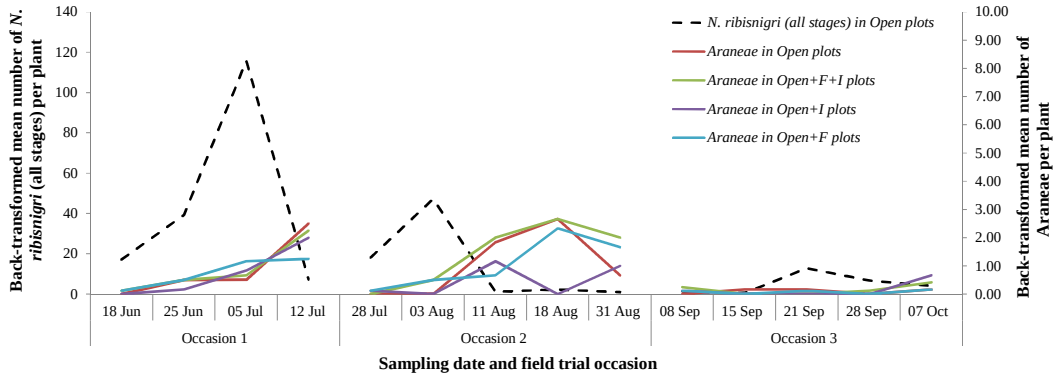
b)



c)



d)



e)

Figure 6.1.38a-e Back-transformed mean number per plant of *N. ribisnigri* and **a)** parasitized aphids **b)** syrphid larvae **c)** Anthocoridae **d)** Neuroptera and **e)** Araneae per plant on each of the open treatments.

Weather

Temperature, humidity and rainfall records were obtained from the University of Warwick, Wellesbourne meteorological station. Figure 6.1.39 shows the mean maximum and minimum temperatures and mean relative humidity recorded during each month. The maximum mean temperature was 23.2ºC (for July) and the minimum mean temperature was 9.8ºC (for June). Mean relative humidity increased gradually through the summer, from 65.86 to 78.70 %.

The highest mean monthly rainfall occurred in August (4.14mm) and the least in July (0.66mm) (Figure 6.1.40).

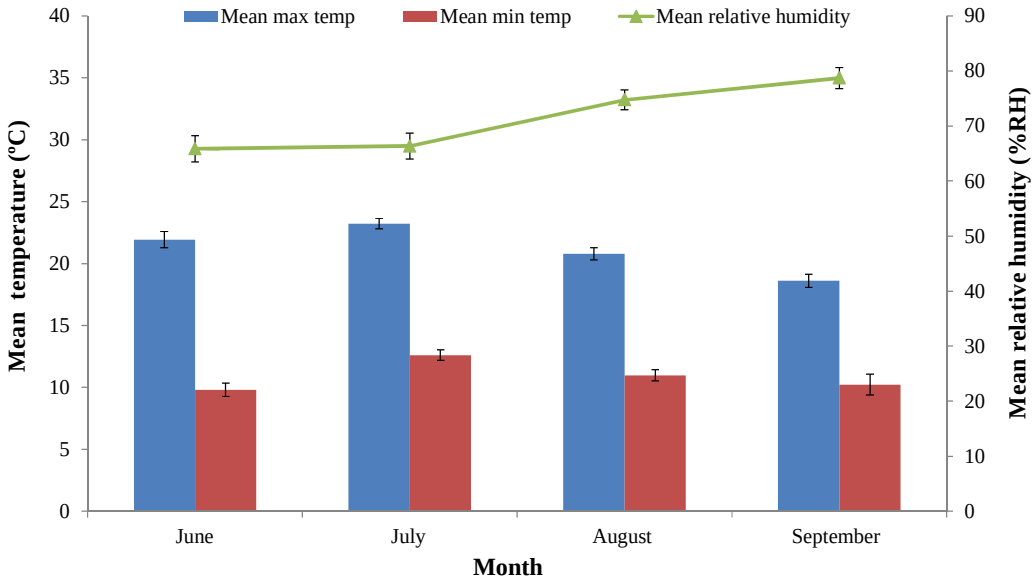


Figure 6.1.39 Mean maximum and minimum temperatures (ºC±SE) and mean relative humidity (%RH±SE) recorded during each month.

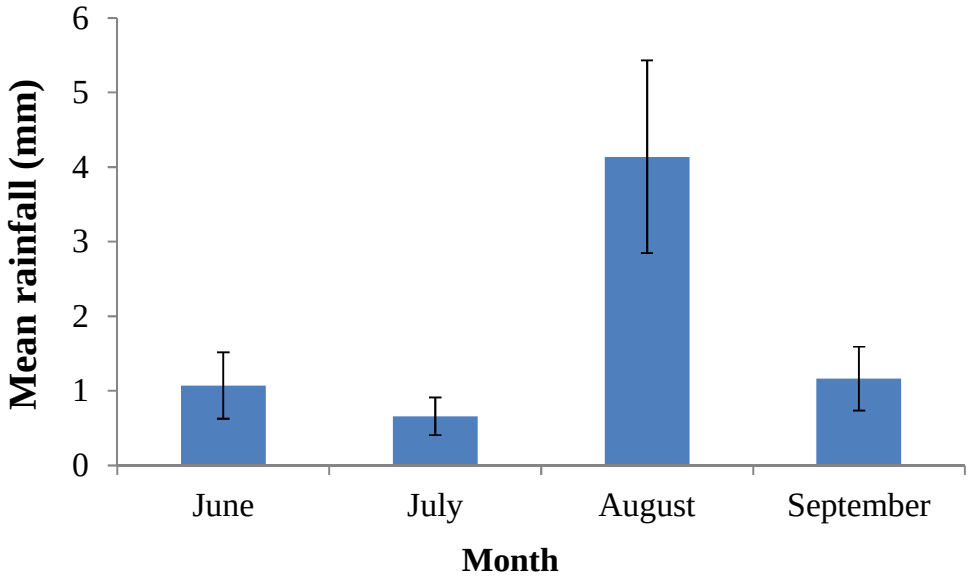


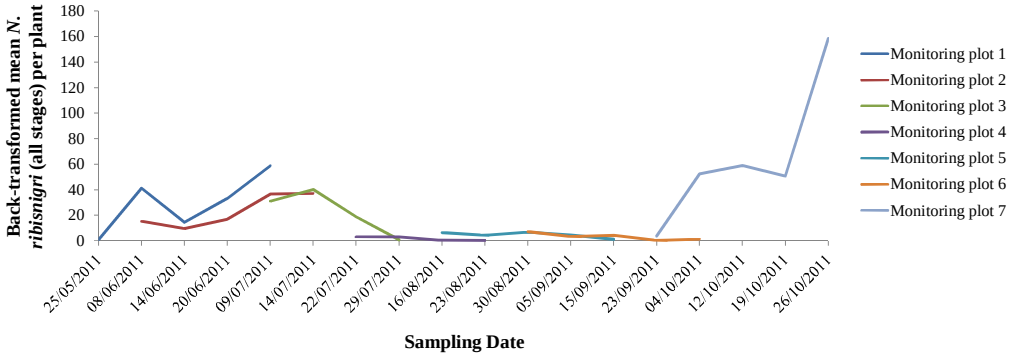
Figure 6.1.40 Mean rainfall (mm±SE) recorded during each month.

6.2 Field trial 2011

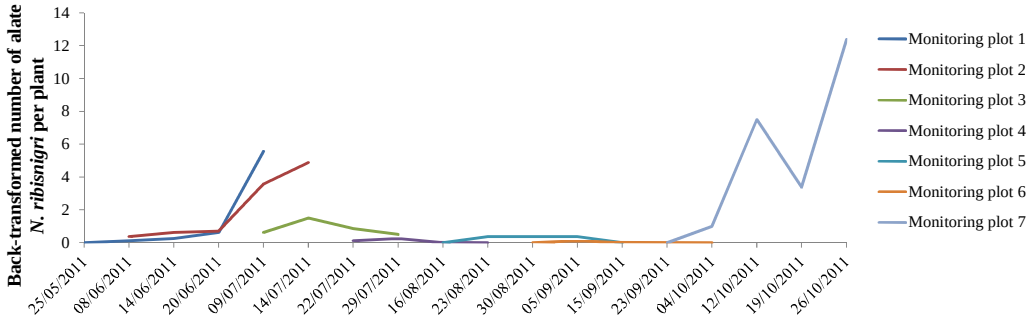
Monitoring plots

Aphids

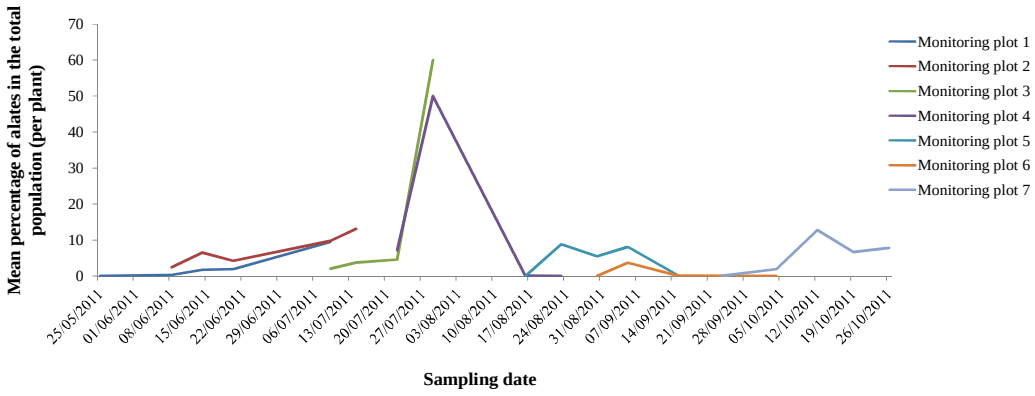
Figure 6.2.7a shows the mean number of *N. ribisnigri* (all stages) per plant on each sampling date for the seven monitoring plots. The largest numbers of *N. ribisnigri* occurred late in the season on 26 October 2011 with a second, smaller, peak on 9 July 2011. Very low numbers of aphids were found between these peaks, following a decline starting on 14 July 2011. Numbers began to increase again from 23 September 2011. This suggests that the mid-summer crash occurred between 14-29 July 2011. The numbers of alates per plant followed a similar pattern (Figure 6.2.7b). Figure 6.2.7c shows the mean percentage of the total population per plant which were alates at each sampling date. This shows that the percentage of alates in the population increased to up to 13% by 14 July 2011 and then up to 60% by 29 July 2011. Following this the percentage of alates decreased to 0% and continued to fluctuate below 13%.



a)



b)



c)

Figure 6.2.7 a-c Back-transformed mean **a)** number of *N. ribisnigri* (all stages) **b)** alate *N. ribisnigri* **c)** percentage of alates in the total population, recorded per plant on each sampling date from May to October 2011 in the seven monitoring plots.

Macrosiphum euphorbiae was present in much higher numbers than *N. ribisnigri*, but it also displayed a mid-summer crash following a peak in numbers on 14 July (Figure 6.2.8).

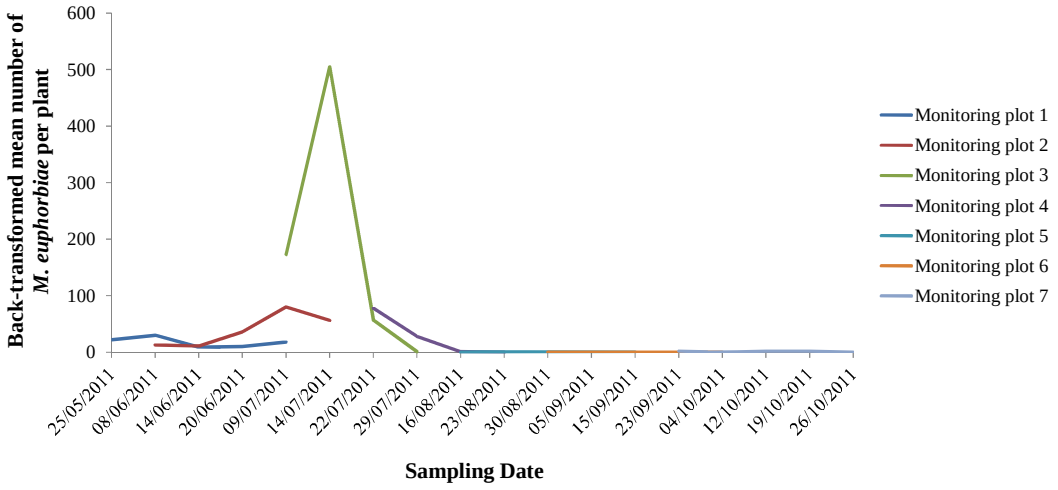


Figure 6.2.8 Back-transformed mean numbers of *M. eurphorbiae* recorded on each sampling date from May to October 2011 in the seven monitoring plots.

Myzus persicae was present in similar numbers to *N. ribisnigri* but displayed erratic changes in abundance, with peaks on 20 June, 14 and 29 July in Plots 2, 3 and 4 respectively (Figure 6.2.9). Like *N. ribisnigri*, numbers also increased from 23 September.

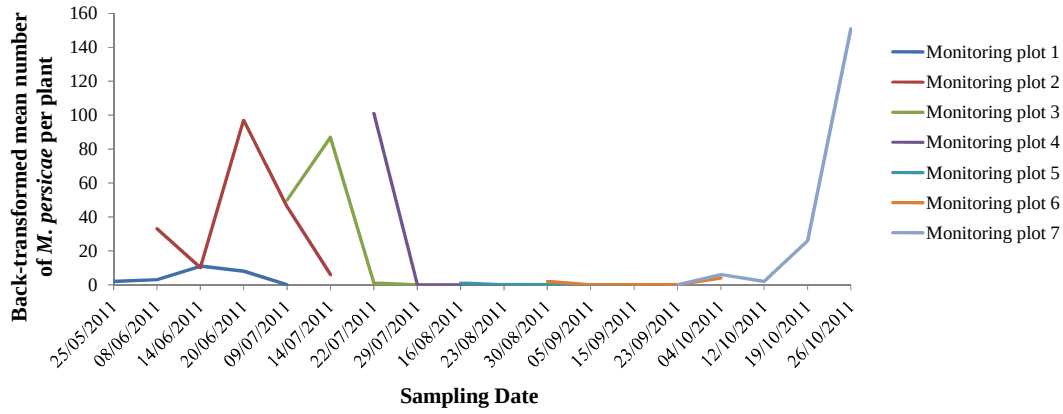


Figure 6.2.9 Back-transformed mean numbers of *M. persicae* recorded on each sampling date from May to October 2011 in the seven monitoring plots.

Natural enemies

Parasitized aphids were the most prevalent natural enemies with a mean of 14.5 parasitized aphids per plant on 9 and 14 July (Figure 6.2.10a). Following this peak in numbers, the mean number of parasitized aphids per plant decreased and remained low for the remaining sampling dates.

Syrphid larvae were observed from 20 June onwards, peaking on 22 July with a mean of 1.14 syrphid larvae per plant (Figure 6.2.10b). The numbers of syrphid larvae were very low and there were no clear fluctuations in numbers.

Aphids infected with entomopathogenic fungi were also counted and the mean number of infections peaked on 14 July, with five infected aphids per plant (Figure 6.2.10c). Infection by entomopathogenic fungi was observed only between 20 June and 29 July. Numbers of Coccinellidae, Anthocoridae and Neuroptera were very low and have not been presented.

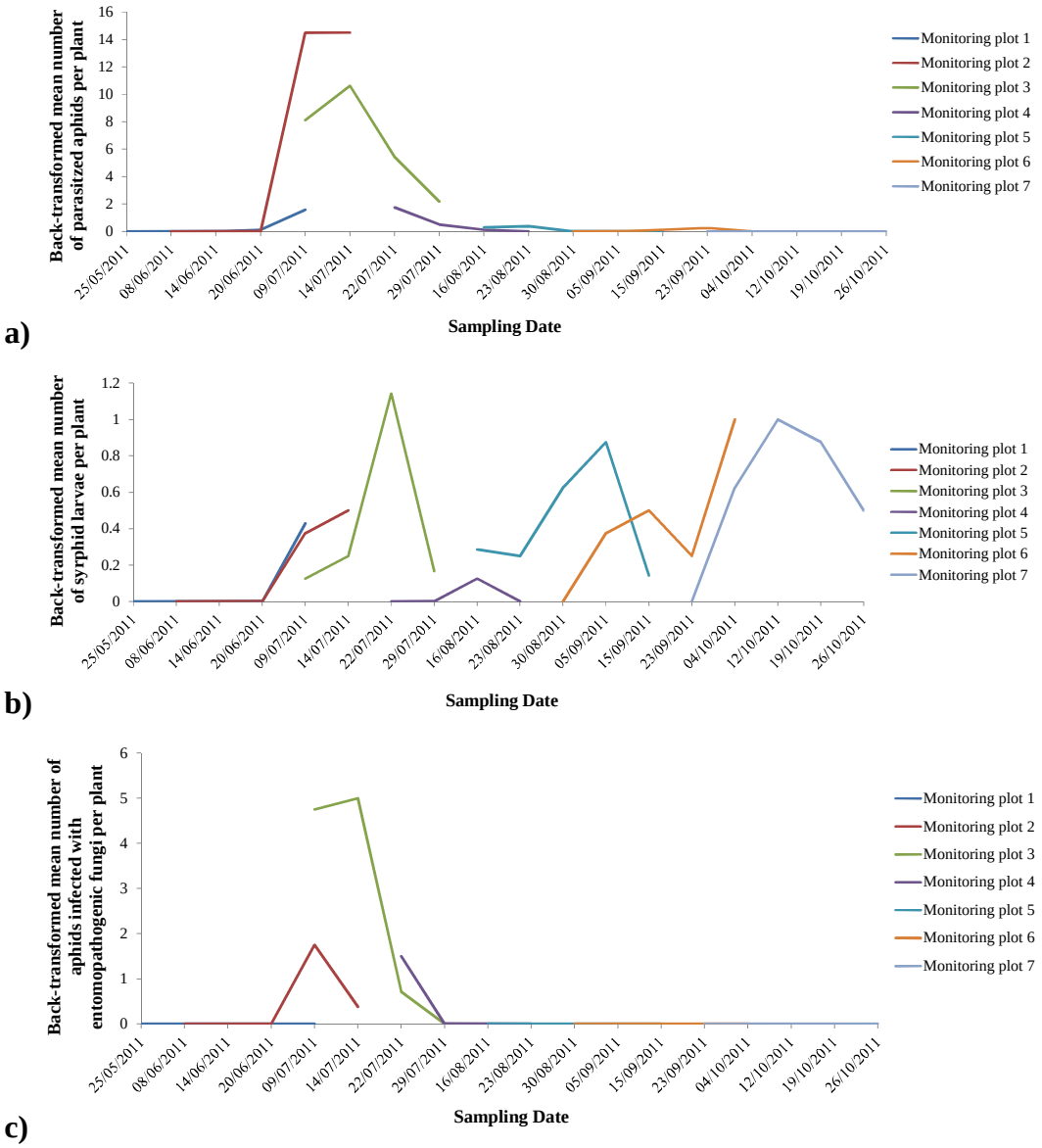


Figure 6.2.10 a-c Back-transformed mean number of **a)** parasitized aphids per plant **b)** syrphid larvae per plant and **c)** aphids infected with entomopathogenic fungi per plant on each sampling date from May to October in the seven monitoring plots.

Water traps

Figure 6.2.11 shows the total numbers of natural enemies found in the water traps near the monitoring plot. Syrphid adults were the most numerous, with a maximum of 80 recorded on 4 August. Maximum counts of Coccinellidae and Anthocoridae were much lower, with 11 Coccinellidae on 29 July and six Anthocoridae on 16

August. Counts of Neuroptera never exceeded one on any sampling date and have not been shown.

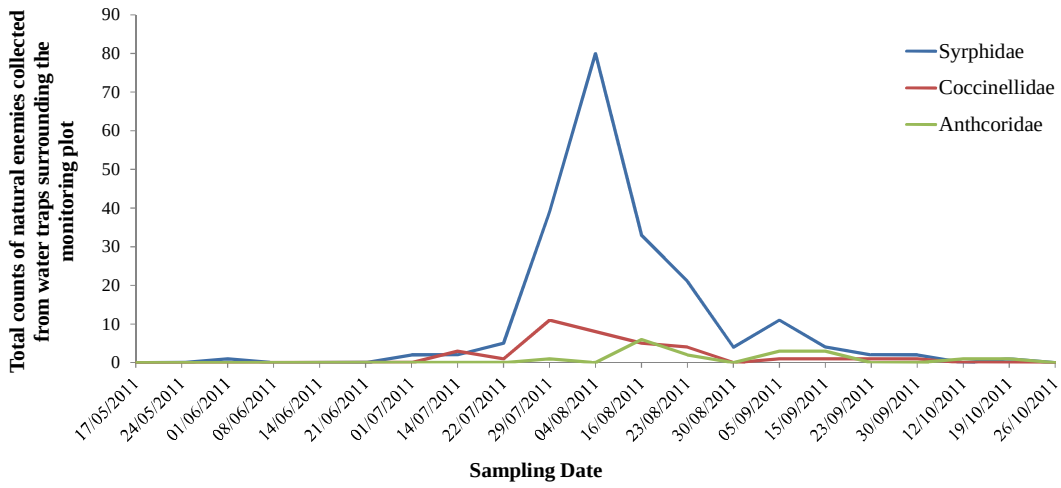


Figure 6.2.11 Counts of Syrphid adults, Coccinellidae and Anthocoridae recorded in monitoring plot water traps.

Field trial 2011- Trial plots

Aphids

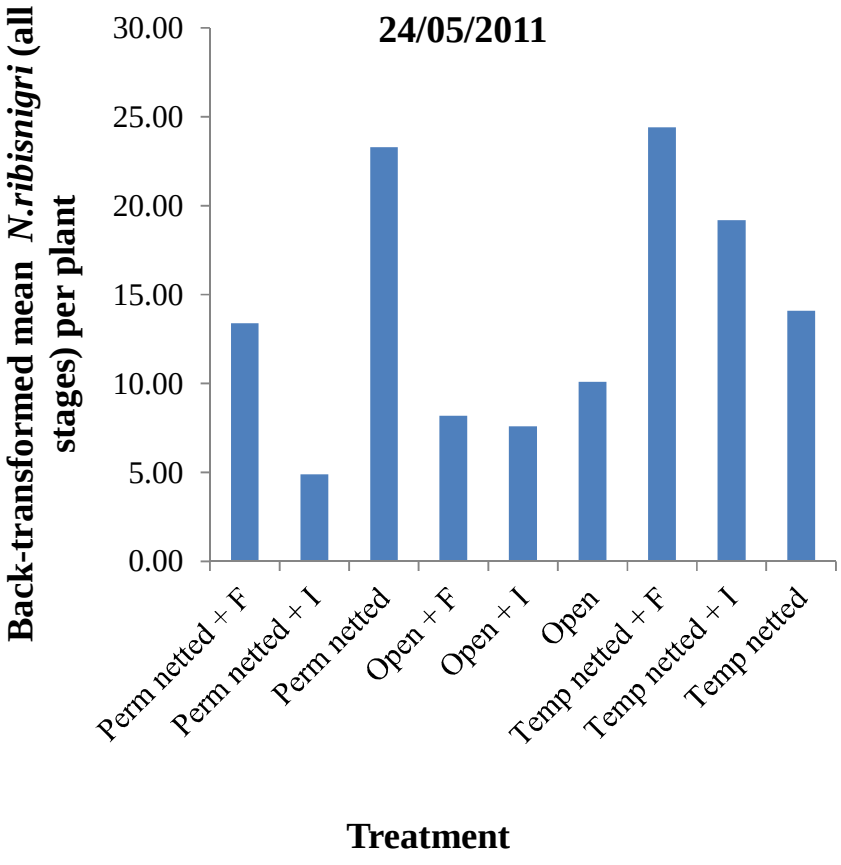
Figure 6.2.12a-e shows the back transformed mean number of *N. ribisnigri* (all stages) per plant on the nine treatments at the end of each field trial carried out in May, June, July, August and September. Only data for lettuce plants inoculated with *N. ribisnigri* were included, as aphid numbers were significantly lower on those plants which had not been inoculated.

Each trial provides an indication of the impact on *N. ribisnigri* from the introduction of natural enemies and entomopathogenic fungi by comparing the numbers of aphids at the end of the trial in temporarily netted plots, with those in permanently netted and open plots.

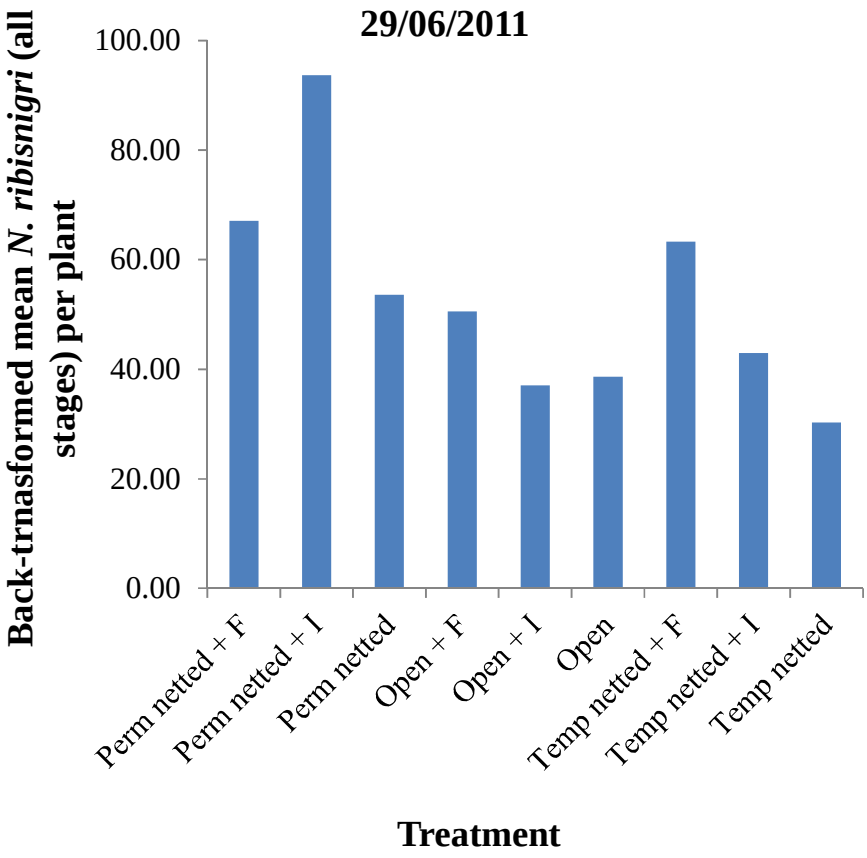
As shown in Figure 6.2.12 b and e, the highest numbers of aphids were found in the June and September trials, reaching 93.7 and 113.30 aphids per plant respectively in netted plots. Unfortunately, the trial during May was severely damaged by hares and the numbers recorded are likely to be lower than from undamaged plants. The lowest number of aphids observed occurred during the trials in July and August which were

infested with means of 3.3 and 1 *N. ribisnigri* per plant respectively, in the open treatments.

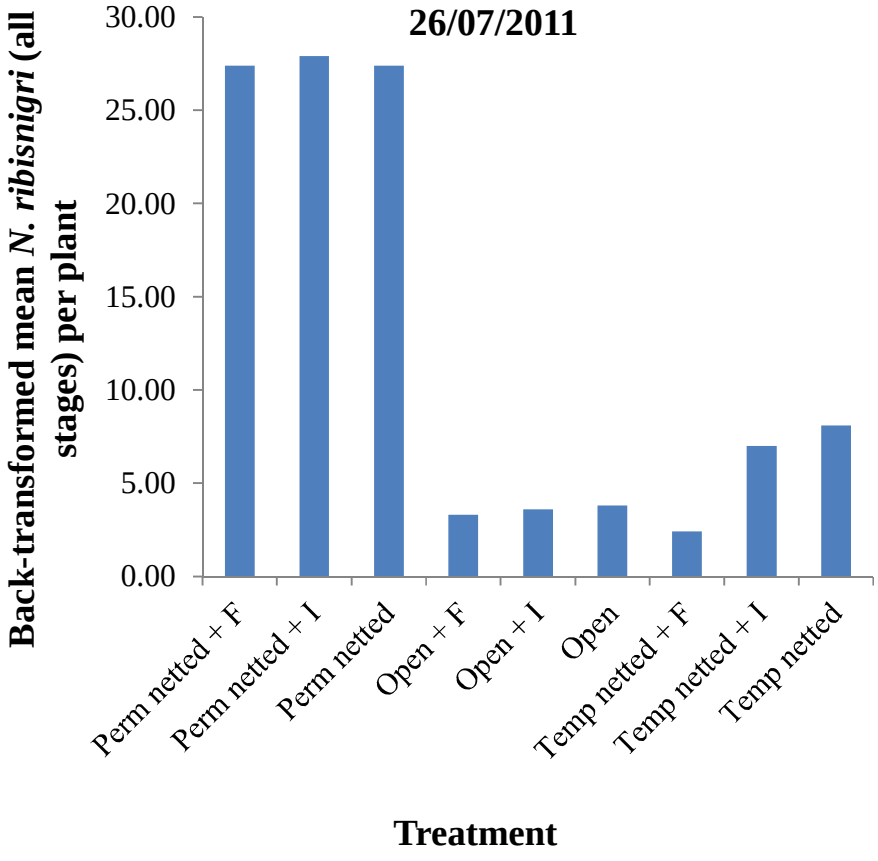
The trial in July had the lowest mean numbers of *N. ribisnigri* per plant in temporarily netted plots. The largest differences between permanently netted and temporarily netted treatments was a decrease of 25 aphids per plant between the Temp Netted+F and Perm Netted+F treatments. The monitoring plots indicated that the mid-summer crash occurred around 14 July and this period was covered by the trial in July.



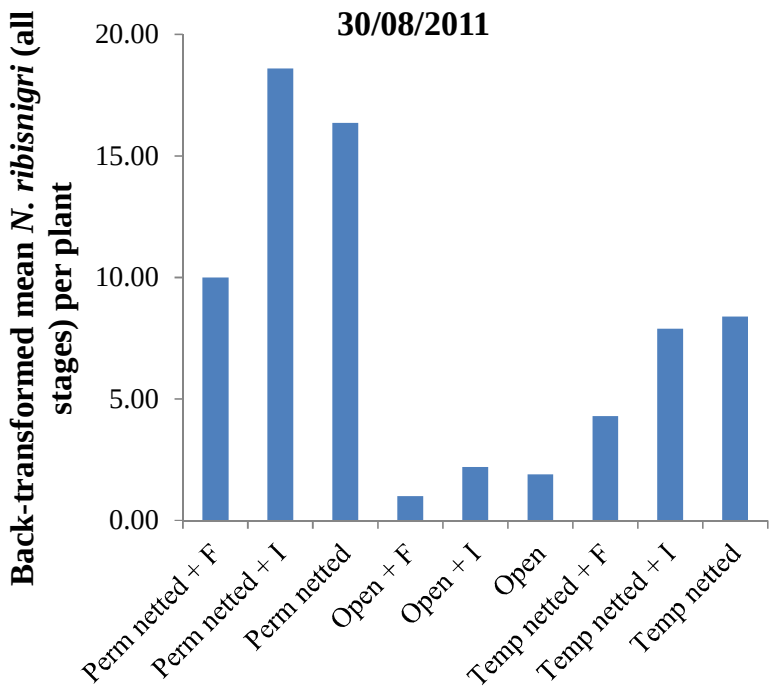
a)


29/06/2011
Back-trnasformed mean N. ribisnigri (all stages) per plant
100.00
80.00
60.00
40.00
20.00
0.00
Perm netted + F
Perm netted + I
Perm netted
Open + F
Open + I
Open
Temp netted + F
Temp netted + I
Temp netted
Treatment

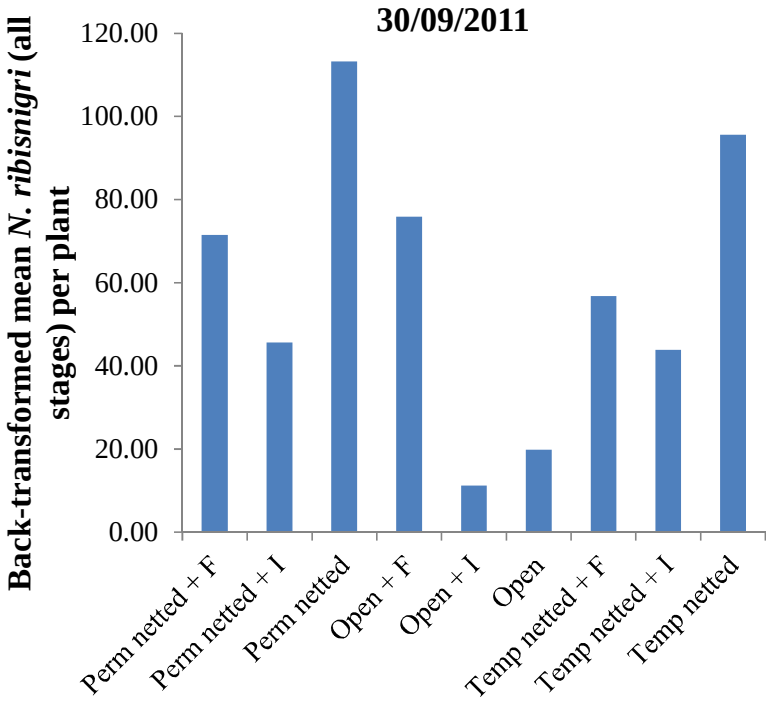
b)


26/07/2011
Back-transformed mean N. ribisnigri (all stages) per plant
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Perm netted + F
Perm netted + I
Perm netted
Open + F
Open + I
Open
Temp netted + F
Temp netted + I
Temp netted
Treatment

c)



d)



e)

Figure 6.2.12 a-e Back-transformed mean number of *N. ribisnigri* (all stages) sampled per plant at the end of each field trial on **a)** 24 May **b)** 29 June **c)** 26 July **d)** 30 August and **e)** 30 September 2011.

An ANOVA was performed on all stages of *N. ribisnigri* per plant in each field trial. As shown in Table 6.2.13 there was an overall effect of field trial month, with June and September having significantly more aphids per plant ($F(4,5)= 111.59$, $p<0.001$). The netting treatment had an effect on the number of *N. ribisnigri* with open plots having significantly less aphids per plant than both temporarily netted and permanently netted plots (Table 6.2.14) ($F(2,40)= 31.30$, $p<0.001$). There were also significantly less aphids in the temporarily netted plots compared to the permanently netted plots.

Table 6.2.13 Transformed mean of *N. ribisnigri* (all stages) per plant from the ANOVA for the effect of field trial month.

Field trial month	**Mean**
May	2.573
June	3.867
July	2.208
August	1.863
September	3.772
LSD	0.2890
d.f.	40

Table 6.2.14 Transformed mean total number of *N. ribisnigri* (all stages) per plant from the ANOVA for the effect of netting treatment.

Treatment	**Mean**
Permanently Netted	3.383
Temporarily Netted	2.927
Open	2.259
LSD	0.2890
d.f.	40

As shown in Figure 6.2.15, there was an interaction between field trial month and netting on the number of *N. ribisnigri* (all stages) per plant ($F(8,40)= 4.22$, $p<0.001$). Only in July and August was there a significant difference between permanently netted plots and both temporarily netted and open plots. Aphid numbers per plant from permanently netted plots were significantly different to those from open plots in July, August and September. No significant effects of fungicide or insecticide application were observed.

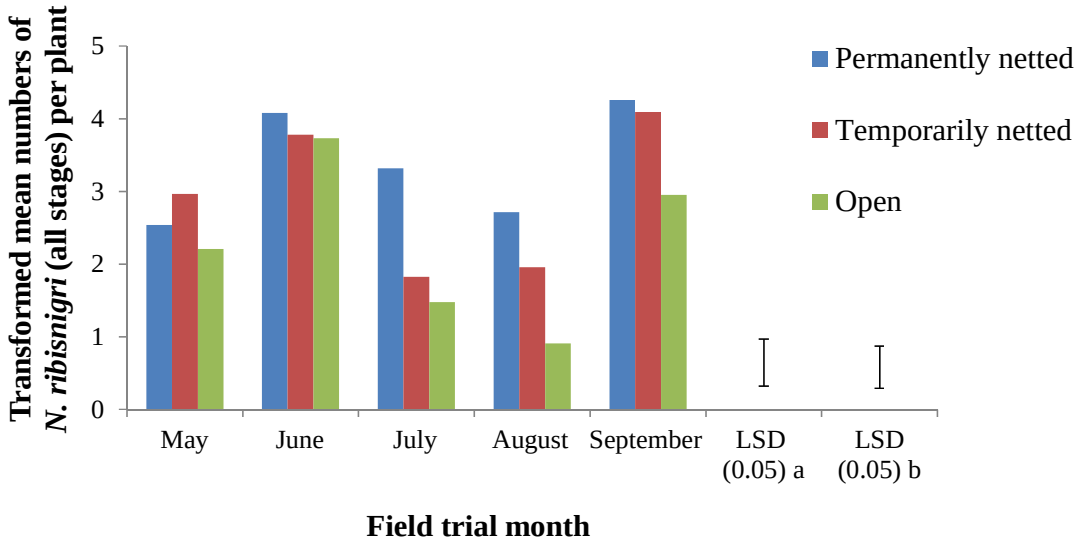


Figure 6.2.15 Transformed mean number of *N. ribisnigri* (all stages) per plant from the ANOVA for the interaction between field trial month and netting treatment. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

When analysing the mean number of alate *N. ribisnigri* per plant, only an effect of field trial month was observed ($F(4,5)= 30.11$, $p=0.001$) (Figure 6.2.16). Alate numbers in field trial months May, July and August were significantly lower than those in June and September

Table 6.2.16 Transformed mean number of alate *N. ribisnigri* per plant from the ANOVA for the effect of field trial month.

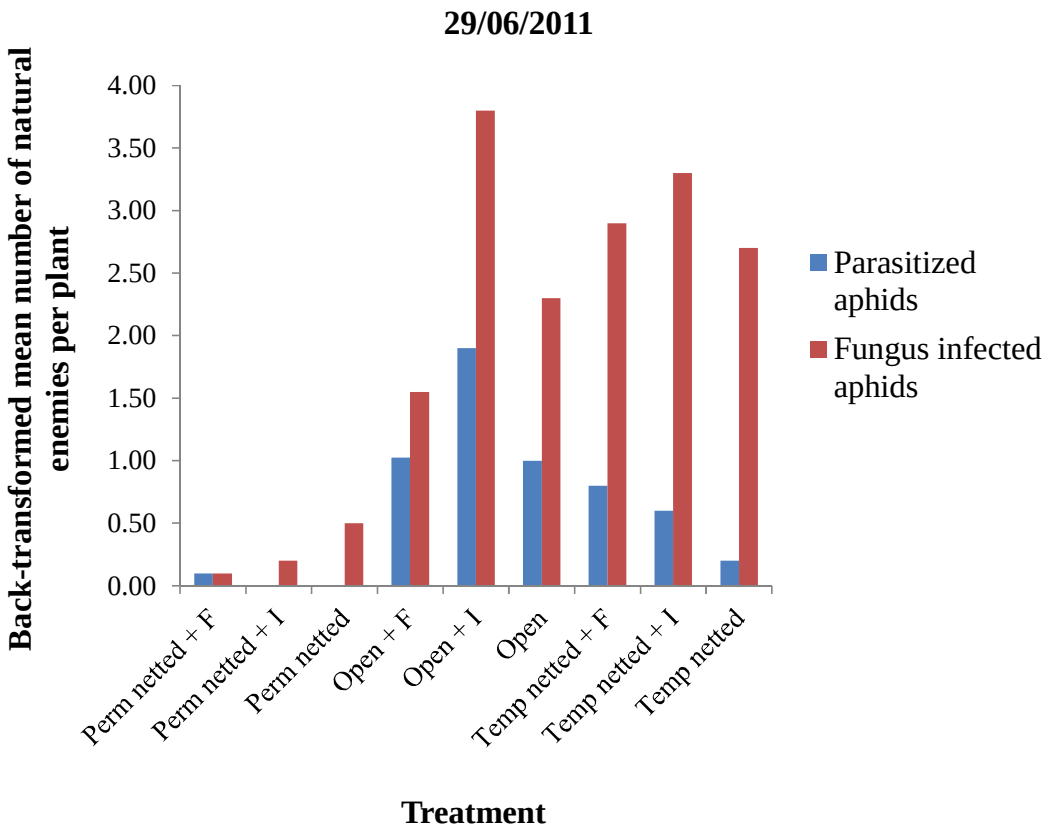
Field trial month	Mean
May	0.170
June	0.501
July	0.125
August	0.193
September	0.496
LSD	0.1228
d.f.	5

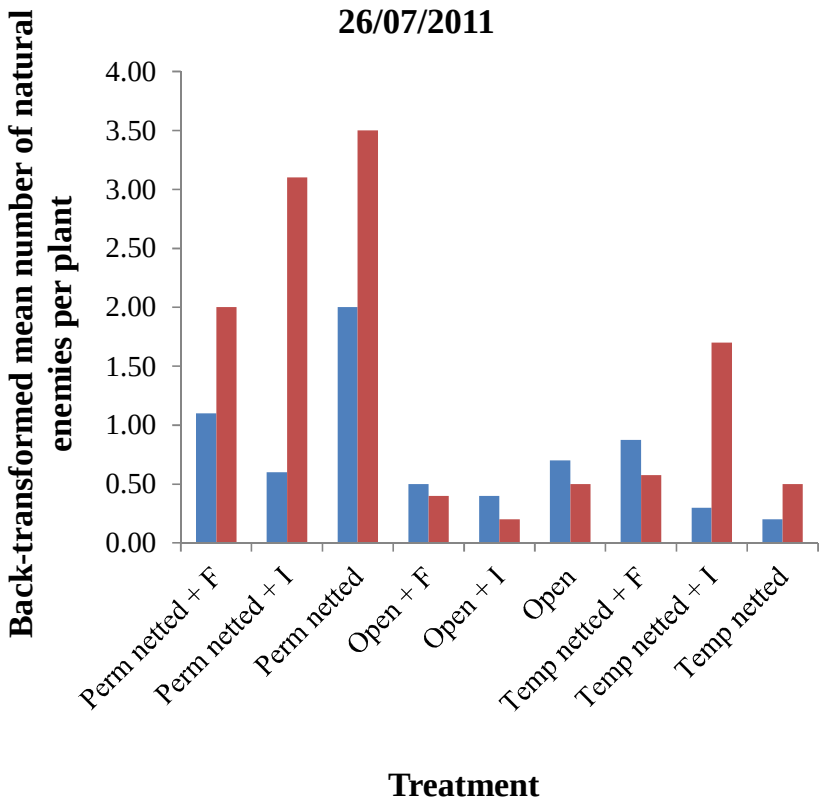
Natural enemies

When considering the numbers of natural enemies found on plants from each of the nine treatments in each field trial month (data on inoculated lettuce plants only) most 'types' of natural enemy were present in very low numbers for all the treatments (mean less than one).

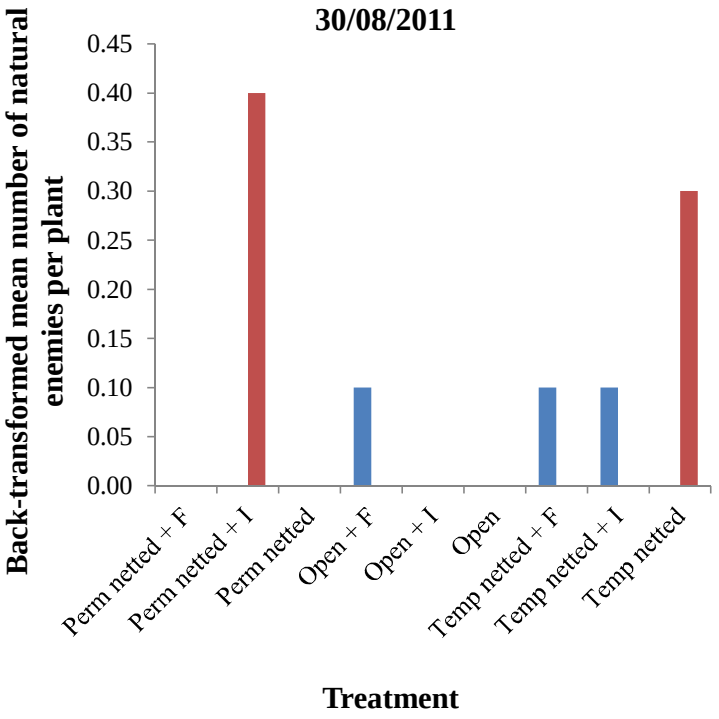
Figure 6.2.17 shows the mean number of parasitized aphids per plant and aphids infected with entomopathogenic fungi per plant which were present in the June-September field trials. No natural enemies or aphids infected with entomopathogenic fungi were recorded during the May field trial. In the June trial (Figure 6.2.17a), more infected and parasitized aphids were observed in the temporarily netted and open plots compared to any other field trial month, whilst in the July trial there were more infected and parasitized aphids in the permanently netted plots. In August and September there were fewer infected and parasitized aphids and they were not present in all of the treatments.

a)

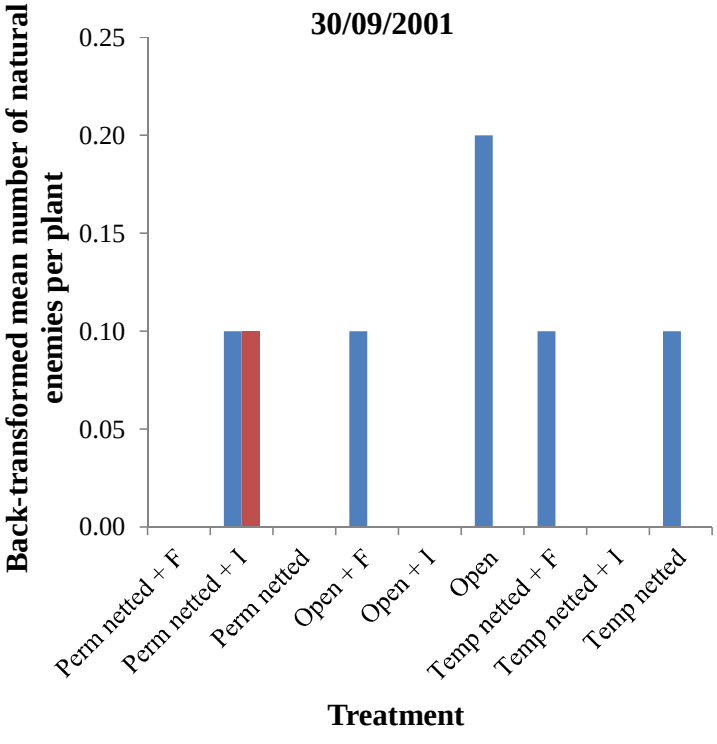

29/06/2011
Back-transformed mean number of natural enemies per plant
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Parasitized aphids
Fungus infected aphids
Perm netted + F
Perm netted + I
Perm netted
Open + F
Open + I
Open
Temp netted + F
Temp netted + I
Temp netted
Treatment


26/07/2011
Back-transformed mean number of natural enemies per plant
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Perm netted + F
Perm netted + I
Perm netted
Open + F
Open + I
Open
Temp netted + F
Temp netted + I
Temp netted
Treatment

b)



c)



d)

Figure 6.2.17 a-d Transformed mean number of parasitized aphids and aphids infected with entomopathogenic fungi per plant sampled from inoculated lettuce plants on **a)** 29 June **b)** 26 July **c)** 30 August and **d)** 30 September 2011.

While the numbers of natural enemies were generally low, an ANOVA was performed on the numbers of parasitized aphids, infected aphids and total natural enemies recorded in each field trial month. Other families of natural enemies recorded, including Anthocoridae, Coccinellidae, syrphid larvae and Araneae, were not analysed individually as the means were less than one due to numerous zero values. No clear relationships were observed between any of the natural enemies and numbers of *N. ribisnigri.*

Parasitized aphids

There was a significant effect of field trial month ($F(4,5)= 20.41$, $p=0.003$) on the number of parasitized aphids per plant (Figure 6.2.18). Significantly more parasitized aphids were observed in June and July.

Table 6.2.18 Transformed mean number of parasitized aphids per plant from the ANOVA for the effect of field trial month.

Field trial month	Mean
May	0
June	0.405
July	0.500
August	0.030
September	0.059
LSD	0.1890
d.f.	5

There was an interaction between field trial month and netting treatment ($F(8,40)= 9.19$, $p<0.00$)) (Figure 6.2.19). The June trial saw significantly more parasitized aphids on temporarily netted and open plots compared with the permanently netted plots, while in July there were significantly more parasitized aphids in permanently netted plots. The May, August and September trials produced fewer parasitized aphids from all netting treatments.

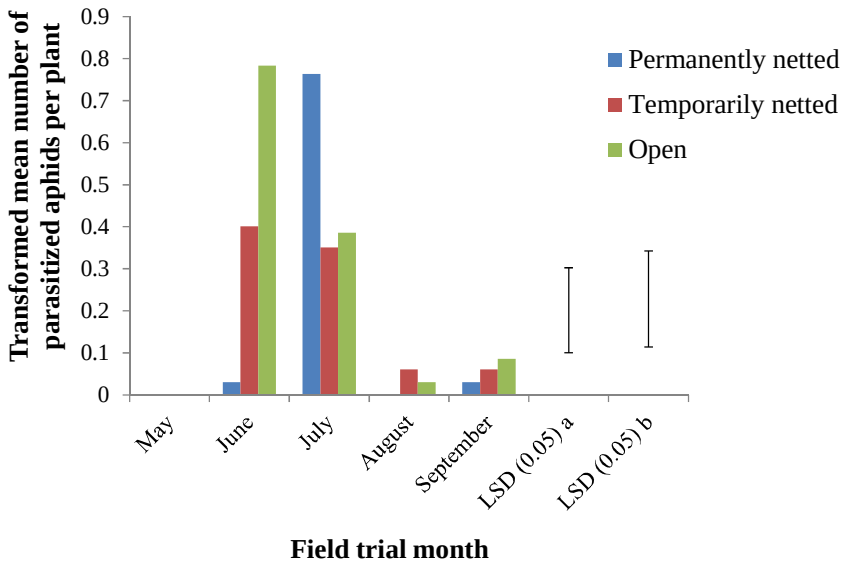


Figure 6.2.19 Transformed mean number of parasitized aphids per plant from the ANOVA for the interaction between field trial month and netting. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

Entomopathogenic fungi

There was a significant effect of field trial month ($F(4,5)= 121.27$, $p<0.001$) on the number of infected aphids per plant (Figure 6.2.20).

Figure 6.2.20 Transformed mean number of aphids infected with entomopathogenic fungi per plant from the ANOVA for the effect of field trial month.

Field trial month	Mean
May	0
June	0.999
July	0.691
August	0.059
September	0.010
LSD	0.1531
d.f.	5

There was an interaction between field trial month and netting treatment on the number of infected aphids per plant (F(8,40)= 13.86, p<0.001) (Figure 6.2.21). The June trial produced significantly more infected aphids per plant on temporarily netted and open plots compared with the permanently netted plots, while in July there were significantly more in permanently netted plots. The May, August and September trials produced fewer infected aphids from all netting treatments. No effect of insecticide or fungicide was observed

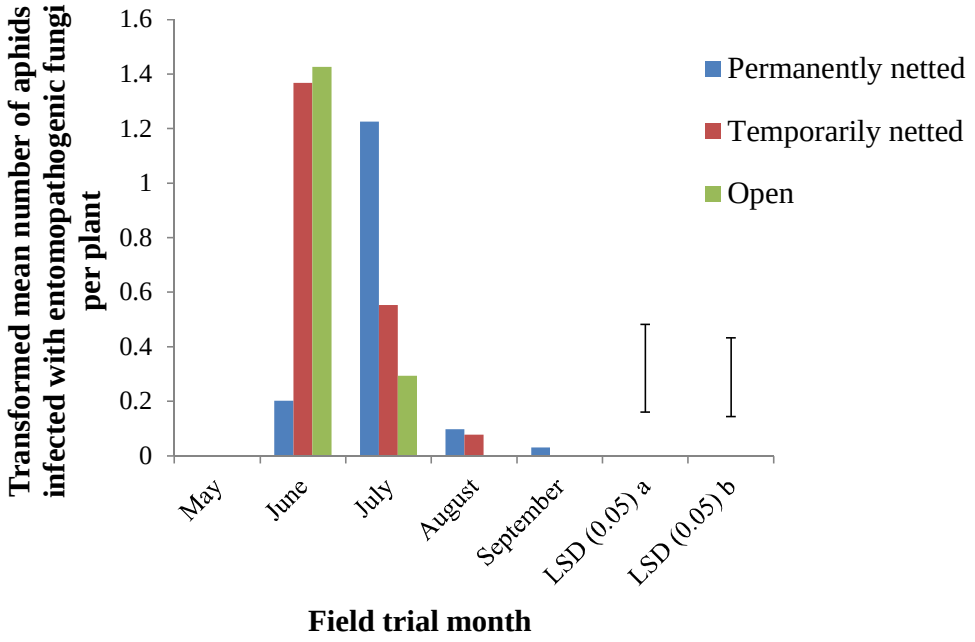


Figure 6.2.21 Transformed mean number of aphids infected with entomopathogenic fungi per plant from the ANOVA for the interaction between field trial month and netting treatment. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

Total number of natural enemies

When the numbers of Anthocoridae, Coccinellidae, parasitized aphids and syrphid larvae were summed there was an overall individual effect of field trial month ($F(4,5)= 23.70$, $p=0.002$), netting ($F(2,40)= 8.39$, $p<0.001$) and treatment ((F(2,40)=3.66, p=0.035)) . Significantly more natural enemies per plant were observed in July (Table 6.2.22). In addition, significantly more natural enemies were observed in open plots compared with permanently netted and temporarily netted

plots as (Table 6.2.23) and there were significantly less natural enemies in plants from insecticide-treated plots than from the control or from fungicide-treated plots (Table 6.2.24).

Table 6.2.22 Transformed mean number of natural enemies from the ANOVA for the effect of field trial month.

Field trial month	Mean
May	0.000
June	0.413
July	0.604
August	0.082
September	0.226
LSD	0.1839
d.f.	5

Table 6.2.23 Transformed mean number of natural enemies from the ANOVA for the effect of netting treatment.

Netting treatment	Mean
Permanently Netted	0.181
Temporarily netted	0.258
Open	0.357
LSD	0.0869
d.f.	40

Table 6.2.24 Transformed mean number of natural enemies from the ANOVA for the effect of treatment.

Spray treatment	Mean
Control	0.303
Fungicide	0.294
Insecticide	0.198
LSD	0.0869
d.f.	40

Interactions were observed between netting treatment and field trial month ($F(8,40)= 8.53$, $p<0.001$) and treatment and field trial month ($F(8,40)=2.87$, $p=0.013$). Figure 6.2.25 shows the interaction between field trial month and netting treatment. In all months except July the highest numbers of natural enemies per plant were found in the open plots. During July there were significantly more natural enemies in permanently netted plots. In June, there was a significant difference in the numbers of natural enemies per plant between all the netting treatments in that month.

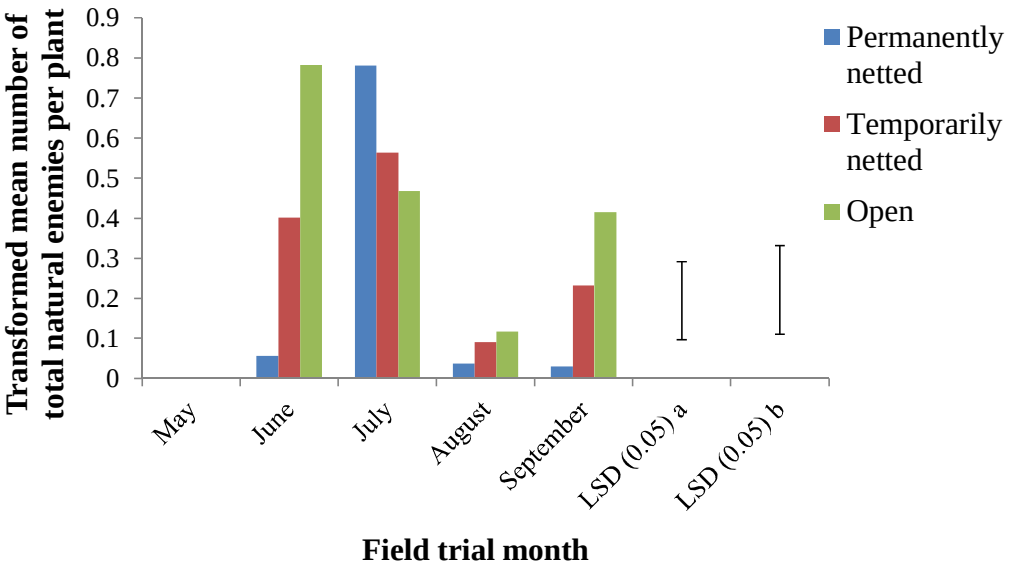


Figure 6.2.25 Transformed mean total number of natural enemies per plant from the ANOVA for the interaction between field trial month and netting treatment. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

Figure 6.2.26 shows the interaction between field trial month and spray treatment. During July and September, the control plots contained significantly more natural enemies than those sprayed with insecticide. In June the opposite effect was observed, with the control having significantly less natural enemies than the plots treated with insecticide. Numbers of natural enemies in the control plots never differed from the plots sprayed with fungicide. Only during July was there a significant difference between fungicide and insecticide treatments.

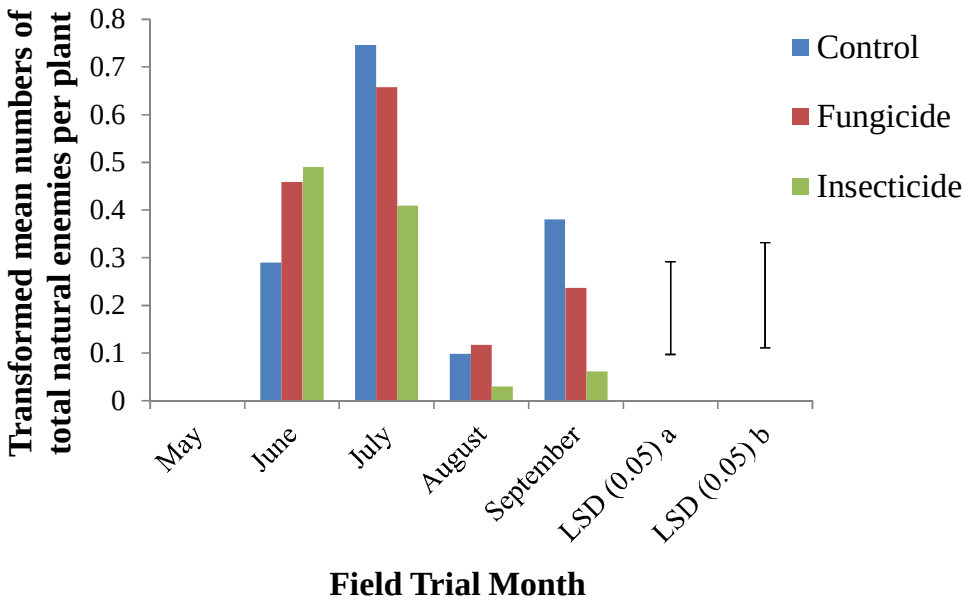


Figure 6.2.26 Transformed mean total number of natural enemies per plant from the ANOVA for the interaction between field trial month and spray treatment. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

Water traps

Figure 6.2.27 shows the total numbers of natural enemies in water traps located near to the field trial. Peak numbers of syrphid larvae occurred around the 29 July (21). Coccinellidae and Anthcoridae were the only other natural enemies captured, but they never exceeded more than 1 or 2 at each sampling date.

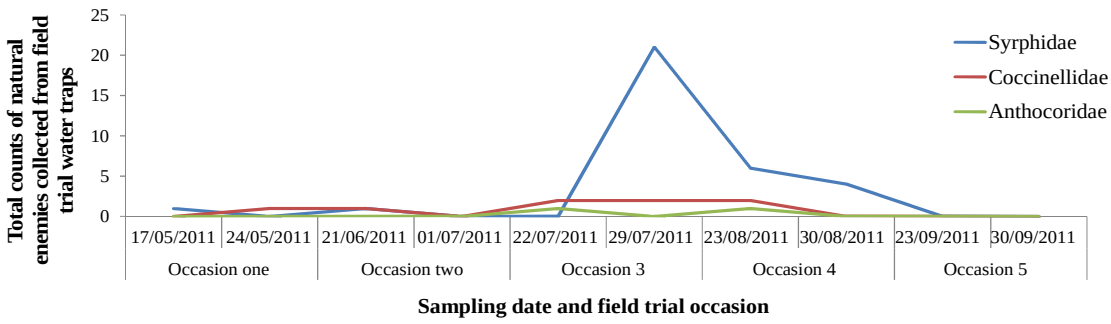


Figure 6.2.27 Total numbers of syrphid larvae, Coccinellidae and Anthocoridae captured in water traps.

When combining data from water traps near to both the monitoring and field trial plots, *Syrphus ribesii* was the most common syrphid species, followed by what was thought to be *Eupeaodes corolla*. *Sphaerophoria scripta* and *Episyrphus balteatus* were also common. Only two coccinellid species were recorded which were *Coccinella 7-punctata* and *Propylea 14-punctata.*

Temperature

Figure 6.2.28 shows the mean temperature recorded from the field trials in June, July, August and September for netted and open plots. The data for the May field trial month has not been included as the Tinytags© malfunctioned.

Higher temperatures were consistently recorded in netted plots compared to open plots with the largest observed difference of 1.18°C in July. The highest mean temperature of 17.27°C was recorded in netted plots during the July trial. The lowest mean temperature (15.49°C) was recorded during the September trial.

Humidity increased gradually each month in both the netted and open plots, with September having the highest humidity. Except for the June trial, humidity was higher in netted plots compared to open plots, with the largest observed difference occurring during August (3.77%).

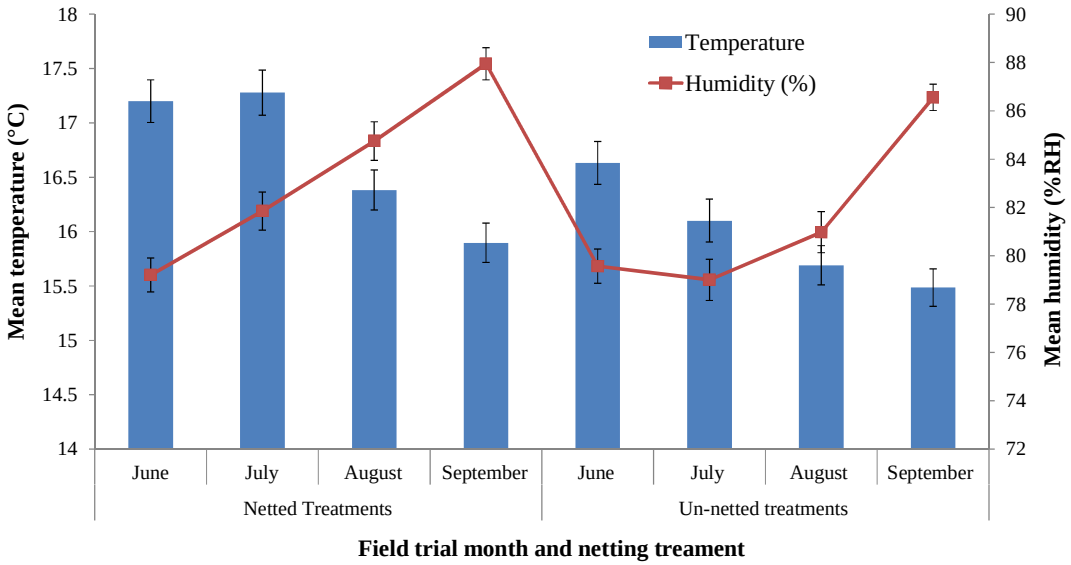


Figure 6.2.28 Mean temperature (°C ±SE) and humidity (%RH ±SE) recorded during each field trial for open and netted plots.

Rainfall

Rainfall records were collected from the University of Warwick, Wellesbourne meteorological station. Figure 6.2.29 shows the mean rainfall and the maximum daily rainfall during each field trial. The highest mean monthly rainfall occurred in August (2.91mm). The maximum rainfall for one day during each field trial month was 10.5, 6.3, 16.7 and 7.3mm on 24 June, 16 July, 24 August and 16 September 2011 respectively.

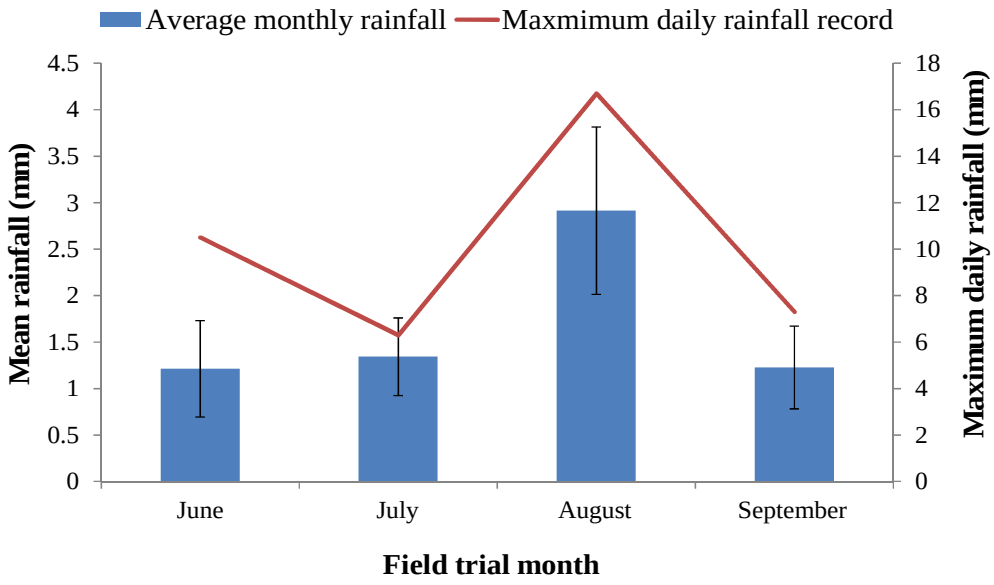


Figure 6.2.29 Mean rainfall (mm ±SE) and the maximum daily rainfall (mm) recorded during each field trial.

Discussion

Field trial 2010

The hypothesis tested during the 2010 field trial, was that if natural enemies play a significant role in regulating aphid populations, then when aphid numbers decline they should do so in the presence of high natural enemy numbers.

Aphid numbers

Sequential planting of the field trial over the summer of 2010 did not allow for continuous monitoring of *N. ribisnigri* numbers in open plots, as the timing of the

plantings did not overlap sufficiently to allow the aphids to naturally distribute and colonise the newly-planted open plots. The control treatment in this experiment also highlighted the need to inoculate future trials with *N. ribisnigri* as uninoculated plots had the lowest number of aphids (Figure 6.1.4b). During field trials Occasion 1 and 3, aphid numbers did not exceed a mean of 2.5 aphids per plant in the control plots. During Occasion 2, aphid numbers peaked on 3 August 2010 with a mean of 24 aphids per plant. This peak may be a result of aphids migrating into the control treatment from the open plots where *N. ribisnigri* numbers were also at their peak.

The use of the open and netted plots was effective in allowing for comparison of aphid populations with natural enemy pressure and populations with reduced natural enemy pressure. The netted plots reduced the numbers of natural enemies, but did not completely exclude them, but the lower natural enemy pressure allowed aphid numbers to continue to increase at each sampling date in each of the Occasions as shown in Figure 6.1.4a. Field trial Occasion 1 was the period of the trial where the r_m was at its peak, as aphids reached their highest numbers, followed closely by Occasion 2 and then Occasion 3. Differences in the numbers of aphids between occasions could be due to temperature where Occasions 1, 2 and 3 had mean temperatures of 16.6, 16.5 and 14.1°C respectively.

The use of netting resulted in 'unrealistically' high counts of alate aphids, and therefore total aphids, as emigration was restricted (Basky, 2003), but it did demonstrate that, generally, the number of alates increased as the total population increased (Figure 6.1.5 and 6.1.6). Considering the percentage of alates in the total population (Figure 6.1.8), generally, during Occasion 1, the percentage of alates in the total population increased as the size of the total population increased. The increase in the number of alates is likely to be due to the effect of population crowding (Müller, *et al.*, 2001) and in later sampling weeks, to a response to the deteriorating condition of the host plant (Karley, *et al.*, 2003). Occasion 2 showed a similar pattern, although some declines in the percentage of alates occurred, even though they were restricted from emigrating. This can be explained by the high number of alates which were observed on the underside of the nets, rather than on the lettuce plants and which dispersed when the nets were removed for sampling, also meaning that the numbers of alates per plant were underestimated. Again Occasion 3 displayed similar fluctuations in the percentage of alates.

In open plots, the positive relationship between alates and non-alate *N. ribisnigri* was not as distinct, as the alates could emigrate and the population was more heavily predated. More variation was also observed for the percentage of alates in the total population (Figure 6.1.11).

Unlike the netted plots, aphid populations in open plots were lower and did not continue to increase with each sampling date, as population declines were observed. The most significant of these declines occurred between the 5 and 12 July 2010, as on 12 July 2010 aphid numbers were much lower than at the previous sampling date, which coincided with the expected timing of the mid-summer crash as observed in other studies (Tatchell, *et al.*, 1998; Collier, *et al.*, 1999; Collier and Harrington, 2001; Karley, *et al.*, 2003). The crash was not observed in netted plots which suggests that netting excluded natural enemies, prevented emigration or altered other factors which are important in causing the decline in aphid numbers. The possible factors causing this decline in open plots will now be discussed.

Emigration

In contrast to the netted plots, mass emigration of alates could have occurred in the open plots. Unfortunately, at the time of the crash, newly transplanted lettuce plants were not available in the field to determine whether colonisation of new plants by emigrating alate *N. ribisnigri* occurred. For emigration to be the cause of the decline, it would need to explain the 'disappearance' of 94% of the population (equivalent to 108 aphids as observed in the open treatment). It would also have to be determined whether it is possible that majority of the 86% of the nymphal population (separated from apterae) recorded on 5 July 2010 could develop into alate adults before the next sampling date on 12 July 2010.

In this study, the percentages of alates in the total population increased up to 5 July 2010 and for some treatments continued to increase to 12 July, becoming 25% of the total population. Therefore, the potential for emigration was increasing prior to the mid-summer crash possibly due to crowding and the deteriorating condition of the host plant as previously discussed. Furthermore, increased alate production can also be induced in aphids following exposure to the aphid alarm pheromone which is released in response to natural enemy attack (Kunert *et al.*, 2005).

From evaluation of the literature, a study on *A. pisum* observed that population declines resulted from nearly all the nymphs produced at high densities developing into alates and emigrating (McVean, *et al.*, 1999). Investigations into the population crash of *Elatobium abietinum* also indicated it was the result of alate emigrants with little influence from natural enemies (Leather and Owuor, 1996).

Various researchers have tried to model the population dynamics of aphid species to explain the causes of population declines, but few have been successful due to the many intrinsic and extrinsic factors which can rapidly influence an aphid population (Kindlmann and Dixon, 2010). However, Kindlmann and Dixon (2010) have recently reviewed the existing models of aphid population dynamics and propose that emigration is the most important factor causing declines in aphid populations, as natural enemies are only effective at reducing aphid populations when populations are small and not at their peak. Another model using the alder aphid as the case organism, showed that density-dependent dispersal could explain the mid-summer crash without including the effects of natural enemies and plant quality (Mashanova, *et al.*, 2008). It is certainly feasible that emigration could be contributing to the decline observed in July.

Natural enemy presence

When considering the effect of natural enemies in the open plots, peaks in the number of syrphid larvae and parasitized aphids coincided with the peak in the number of aphids, which was then followed by the aphid crash. A strong positive relationship was observed between the number of *N. ribisnigri* (all stages) per plant and the number of parasitized aphids per plant on 5 July 2010. The relationship suggested that the number of parasitized aphids increased with an increasing population of *N. ribisnigri.*

In California, syrphid larvae composed over 85% of the total number of predators recorded in various studies and have been determined as one of the most important natural enemies suppressing *N. ribisnigri* populations in organic lettuce production (Smith and Chaney, 2007; Smith, *et al.*, 2008; Hopper, *et al.*, 2011). Previous research has also identified a range of parasitoids for which *N. ribisingri* is the primary host. In Spain this includes braconid wasp species: *Aphidius hieraciorum*, *Aphidius ervi*, and *Aphidius colemani* (Nebreda, *et al.*, 2005). In the UK, *Aphidius*

ribis, *Aphidius matricariae* and *Aphelinus asychis* have been recorded to parasitise *N. ribisinigri*, as well as other species of lettuce aphid, while *Monoctonys paludum* specifically parasitized *N. ribisnigri* (Griffiths, 1960). In the present study, only total parasitism (all aphid species) was recorded and therefore the numbers of parasitized *N. ribisnigri* are unknown. Thus the 'importance' of parasitoids in regulating *N. ribisnigri* may be overestimated.

It is likely that syrphid larvae and parasitized aphids peaked at the same time as the aphid population because natural enemies (hoverfly adults and parasitoids) are attracted to the high densities of prey, which has been observed in other studies on Coccinellids and natural enemy communities (Ives, *et al.*, 1993; Donaldson, *et al.*, 2007; Chacón and Heimpel, 2010). This demonstrates the dynamic relationship between predator and prey, where aphid numbers are determined by, and determine, the numbers of natural enemies.

The present study also indicated that generalist predators such as spiders could also have an important role in regulating aphid numbers, in addition to more specialised predators, as they were generally present throughout the entire trial period in all plots. Other studies have recognised the importance of generalist predators, including spiders and ground beetles, in reducing aphid populations (Edwards, *et al.*, 1979; Lang, 2003; Schmidt, *et al.*, 2004). Predators such as Neuroptera and Anthocoridae were present later in the summer following the aphid crash, indicating that they were unlikely to have contributed significantly to the decrease in the *N. ribisnigri* population during July.

In this study, the destructive sampling method used to determine the presence of mobile predators could have resulted in an underestimation of their activity, as they could have escaped during the sampling process or when awaiting sampling in the cold store. Schmidt (2008) also noted that destructive sampling and field counts underestimated the numbers of mobile predators and highlighted the need to implement other monitoring methods such as sticky traps and water traps.

While experimental evidence from predator cage exclusion experiments supports the suppression of aphid populations by natural enemies (Frazer, *et al.*, 1981b; Basky, 2003; Brosius, *et al.*, 2007a), the issues surrounding the use of cages (e.g. altering microclimate and emigration) has led some researchers to conclude that they are

unsuitable for the assessment of predation, which then leaves little empirical evidence (Kindlmann and Dixon, 2010; Ameixa and Kindlmann, 2011). Furthermore, while modelling and meta-analysis studies have also supported the role of natural enemies in suppressing aphid populations (Tamaki and Long, 1978; Skirvin, *et al.*, 1997; Diehl, *et al.*, 2013), other models have observed little effect, as discussed. A study combining both field observations of predator consumption and estimations of aphid loss from a demographic model also suggested that the natural enemy complex of the mealy plum aphid (*Hyalopterus pruni*) was unable to cause the mid-summer crash (Mills and Latham, 2009). Therefore, the role of natural enemies is tentative.

Weather

While temperatures were not recorded specifically within the netted and open plots, the data collected from a nearby meteorological station did not indicate any extreme temperature or rainfall events which might have resulted in the aphid decline. Mean minimum and maximum temperatures were between 9.8 and 23.2 °C, with a relative humidity of approximately 70 %. July also saw the lowest maximum daily rainfall and the lowest mean monthly amount of rainfall compared to the values for June, August and September, where no population declines were observed. As no rainfall was recorded between 5 and 12 July 2010, the duration and intensity of rainfall can also be ruled out as a contributing factor towards the population crash, which other studies have observed to result in aphids being lost from host plants (Mann, *et al.*, 1995). Mann *et al.* (1995) also observed that wind duration, in addition to rainfall, could also dislodge aphids from a plant and as this was not measured in this study it cannot be ruled out as a possible factor. If weather events were responsible for the mid-summer crash, the absence of a crash in the netted treatments can be explained by the covers protecting the crops.

Spray treatments

While the use of netting was effective in reducing natural enemy pressure, the use of insecticide and fungicide did not reduce natural enemy numbers or the number of aphids infected with entomopathogenic fungi as planned. Furthermore, the effects of these pesticide applications were not consistent between treatments and were sometimes impossible to explain. For example, the lowest numbers of total natural enemies and parasitized aphids were observed when no spray treatments were used

or when they were combined, but only in netted plots, suggesting that an application of either the insecticide or a fungicide alone were beneficial. Smith *et al.* (2008) also attempted to use an insecticide to specifically suppress numbers of syrphid larvae, again this approach was 'ineffective' but did suppress other predators including minute pirate bugs (*Orius* sp.), ladybird beetles (Coccinellidae), dwarf spiders (Linyphiidae), brown lacewings (Hemerobiidae) and rove beetles (Staphylinidae) in two of the five trials.

This preliminary study indicated possible refinements of the methodology to be used for the 2011 field trial.

Field trial 2011

The hypothesis tested in the 2011 trial, was that if natural enemies and infection by entomopathogenic fungi played a significant role in regulating aphid populations, then the month where the largest decrease was observed between numbers in permanently netted treatments and temporarily netted plots (once the latter were uncovered) would occur during the same period as the mid-summer crash as determined in the monitoring plot, in synchrony with high natural enemy numbers.

Aphid numbers

Only field trial months July and August displayed a significant difference between permanently netted plots and temporarily netted and open plots (Figure 6.3- reproduced from the Results section), where aphids were reduced to very low numbers. This suggests that the factors which were excluded/prevented by netting were effective again as soon as the netting was removed and provided natural aphid control. The largest reduction in aphids in the temporarily netted plots, when compared with the netted plots, occurred in July, coinciding with the mid-summer crash as identified in the monitoring plots (between 14- 22 July 2011). A large decrease was also observed in September, although aphid numbers still remained high.

The decrease observed between netted plots and temporarily netted plots does not take into account the fact that the aphids in the netted plots could not emigrate throughout the trial period. Therefore, the observed decrease may be slightly

overestimated, as numbers in the netted plot may have been lower if emigration could have occurred.

The possible factors causing the mid-summer crash during July will now be discussed.

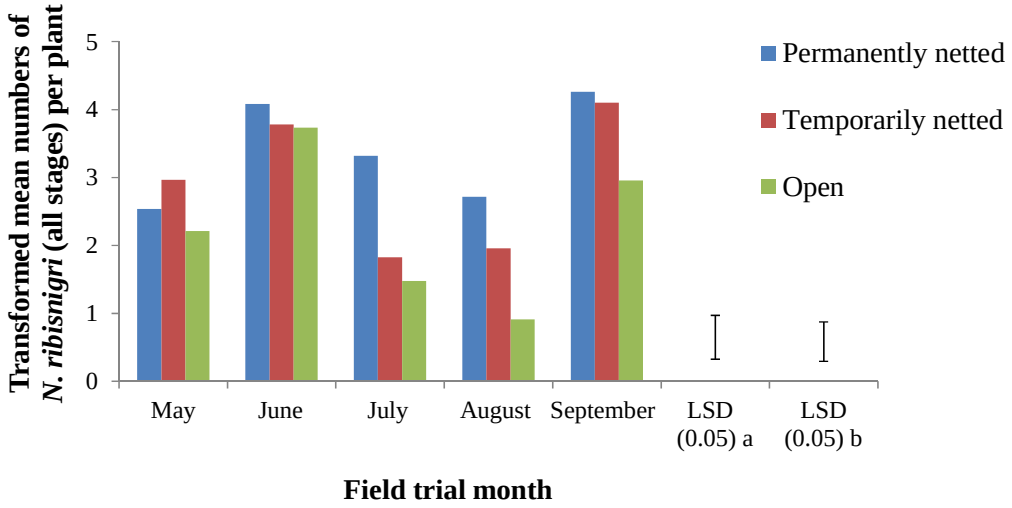


Figure 6.3 Transformed mean number of *N. ribisnigri* (all stages) per plant from the ANOVA for the interaction between field trial month and netting treatment. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

Emigration

Prior to the mid-summer crash which occurred between 14- 29 July 2011, the percentage of alates in the total population on the monitoring plots was increasing and reached 13% on 14 July 2011 (Figure 6.2.7c). Therefore as with the 2010 trial, the potential for emigration was increasing prior to the crash, which is likely to be in response to the crowding effect as discussed. It is less likely that emigration occurred in response to poor host plant quality in this trial, as the decline was observed on all plots which contained plants of different ages.

Alate numbers in the July field trial plot reached a maximum of 4, 11 and 9% of the total population in netted, open and temporarily netted plots respectively. While the numbers of aphids were much lower during this trial year, studies investigating the

thresholds from crowding have observed that as little as two crowded aphids over a one-minute duration can result in the production of alate offspring (Johnson, 1965; Lees, 1967). This demonstrates that emigration can be triggered by very low numbers of aphids per plant.

During July, the crash represented an 87% decrease in aphid numbers in open plots compared to netted plots in the field trial. As aphid numbers were generally lower, this percentage decrease was equivalent to a reduction of only 24 aphids per plant. While this number of aphids could have disappeared as a result of emigration it cannot be confirmed using data from this study. Future experiments should implement methods to predict future emigration, for example by counting the number of 4th instar nymphs with wing buds. For some aphid species aerial sampling can also be used to monitor alate numbers but unfortunately *N. ribisnigri* is 'trap shy' (Collier, *et al.*, 1999) and relatively few are captured in water traps and suction traps.

During the study no increases in aphid numbers were observed in the monitoring plots to suggest that re-colonisation by emigrating aphids occurred.

Natural enemy presence

In the monitoring plots, destructive assessment of the lettuce plants around the time of the mid-summer crash showed that parasitism (of all aphid species) was at its peak, as was the number of aphids infected with fungus. Data from water traps indicated that ladybirds and hover flies were present but did not reach peak numbers until late July/early August, following the aphid decline.

When comparing the numbers of syrphid larvae per plant in monitoring plots (during destructive sampling), with the numbers of syrphid adults captured in water traps at each sampling date, the pattern of syrphid larvae activity on the plants can be explained (Figure 6.4). At the beginning of July, the numbers of syrphid larvae per plant increased in the monitoring plots as they fed on aphids and other prey. The larvae then pupated and in early August and adult syrphids emerged from the pupae. The syrphid adults then laid eggs near colonies of prey, which hatched to produce more larvae, which were then observed on the plants in the monitoring plots around mid-August. The relationship between the counts of Coccinellidae and Anthocoridae

in water traps and those on plants in the monitoring plot could not be determined as less than one per plant was recorded in the monitoring plots.

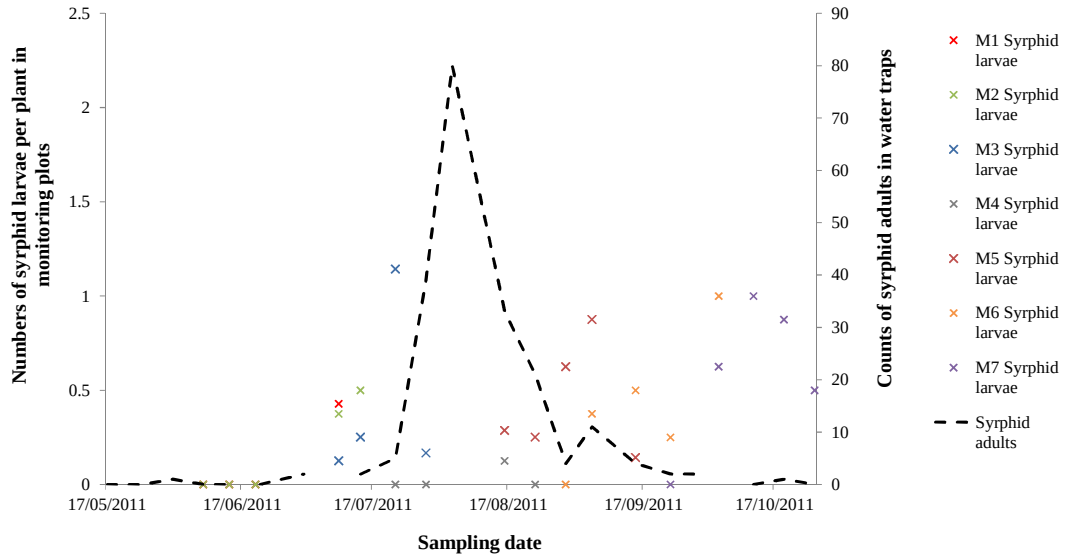


Figure 6.4 The number of syrphid larvae per plant recorded in the seven monitoring plots (M1-M7) and the numbers of syrphid adults captured in water traps at each sampling date.

When considering the role of the natural enemies identified in the field trials in contributing to the population decline, the number of parasitized aphids (Figure 6.2.19), total natural enemies (Figure 6.2.25) and infection by entomopathogenic fungi (Figure 6.2.21) were highest in the field trials during June and July when compared to May, August and September. While natural aphid control was observed in July when the mid-summer crash was observed, little control was observed in June, although natural enemy numbers were also high, which cannot be explained (Figure 6.3).

When aphid numbers declined during the July trial, the temporarily netted plots had only been uncovered for one week, providing natural enemies with only a short period of time to suppress aphid numbers. When considering which natural enemies could respond in this time period, it was concluded that only mobile predators which consume aphids would result in immediate suppression, for example Coccinellidae, Anthocoridae and syrphid larvae. While water traps indicated that Coccinellidae and Anthocoridae were present, very low numbers were recorded on the plants sampled,

suggesting they did not play a significant role in regulating aphids in the trial during the mid-summer crash. Syrphid larvae were also present but at even lower numbers on the plants.

Underestimations of the influence of natural enemies may have resulted from the short trial period used. It has been suggested that crops, such as lettuce, with short growing season may provide insufficient time for natural enemies to increase sufficiently to provide high levels of control (Nunnenmacher and Goldbach, 1996). Therefore, when considering the field trial, the short trial period of two weeks used in this study, may not have provided enough time for natural enemies to build up and achieve control which is why natural enemy numbers appeared to be higher in the monitoring plots on 14 July 2011 (which had been in the field for longer) with up to 14.5 parasitized aphids per plant and 5 fungus-infected aphids per plant, compared with the field trial plots where 2 parasitized aphids per plant and 3.5 fungus-infected aphids per plant were recorded.

Parasitoids appeared to be one of the most prevalent natural enemies during 2011 and it is likely that their influence was also underestimated as the larvae of parasitoids require >1 week to develop before mummies occur (Gutiérrez-Ibáñez*, et al.*, 2007). Therefore, some aphids may have been parasitized during the assessment but were not counted as they had not yet developed into mummies. Underestimations and overestimations may have also been made with the regard to the influence of predators, as this study assumes that when predators are present they predate and suppress aphid populations, which may not be the case. It is also possible that due to the low number of plants sampled in the trial that the numbers of natural enemies were underestimated by their simply not being present on the plants which were assessed. It is therefore important, that future work develops new methods to monitor and measure the influence that natural enemies have in suppressing aphid populations in the field.

When looking at data from water traps situated around the field trial, counts of natural enemies were low around the time of the mid-summer crash in the experimental plots. The only observed peak was the 21 syrphid adults (non-predatory stage) recorded on 29 July 2011. While this cannot be compared directly with the presence of syrphid larvae on the plants in the field trial, because few syrphid larvae

were observed, it does coincide with the peak observed in the water traps in the monitoring plot as shown in Figure 6.4. While in both the monitoring and field trial plots, numbers of hoverfly adults peaked following the aphid decline, only a small number of their larvae (maximum of 1.1 per plant) were observed in 2011 compared to the numbers observed during 2010 (maximum of 4.75 per plant) meaning their effect on the aphid population could not be theorised. These observations could suggest that the plants during the 2011 trial were not suitable for oviposition, due to the low number of aphids present. Previous studies have shown that the size of an aphid infestation can influence the level of oviposition by syrphid females, with different species having a different preferred size of aphid infestation (Chandler, 2008). In this study *S. ribesii* was the most common species and Chandler (2008) found that this species preferred to oviposit on brassica crops with infestations of approximately 2000 *B. brassicae* per plant. As aphid numbers were much lower in the present trial, *S. ribesii,* while present, may have chosen to oviposit elsewhere. Determining the density of *N. ribisnigri* infestations that would be chosen for oviposition by common syrphid species would be valuable for interpreting data from field trials such as these.

Unexpectedly, during July, the highest levels of parasitism, numbers of natural enemies and aphids infected by entomopathogenic fungi were observed in the permanently netted treatments (Figure, 6.2.19, 6.2.21 and 6.2.25), indicating that netting did not completely exclude natural enemies during the trial and may have also promoted the development of entomopathogenic fungi. It is possible that natural enemies and parasitic wasps were enclosed in these plots when they were covered. A study by Basky (2003) showed that predator exclusion techniques prevented emigration of natural enemies and resulted in higher numbers of natural enemies in caged plots compared to open plots. As in the present study, Basky observed that aphid numbers were still higher in the netted plots despite the higher numbers of natural enemies, which suggests that the rate of aphid reproduction must have been higher than the rate at which aphids were consumed or parasitized by natural enemies.

The higher levels of infection by entomopathogenic fungi in netted plots during the July trial could be due to the effect of netting, which resulted in a 1°C increase in temperature and a 2.9% increase in humidity, which may have been optimal

conditions for the development of entomopathogenic fungi compared to the open plots. As aphids infected with entomopathogenic fungi were observed at low levels on the plants in the open plots, temporarily netted plots and monitoring plots it suggests that entomopathogenic fungi did not have a major role in suppressing aphid numbers in the trial but did contribute. Their low occurrence could be due to the low relative humidity during July which was below the 90-93% required for entomopthoralean fungi to be effective (Völkl, *et al.*, 2007).

Weather

The average temperature during July in open plots was 16°C with a humidity of 79%, both lower than the recordings during June. The July trial received an average of 1.3 mm of rainfall and had the lowest recorded maximum daily rainfall of 6.3 mm. Therefore, no extreme weather events occurred during the trial which could explain the aphid crash. However, as previously discussed, the duration and intensity of rainfall and wind was not recorded and cannot be ruled out as a possible contributing factor towards the population crash.

Spray treatment

While insecticide and fungicide treatments during 2010 were ineffective and provided unexplained effects, during 2011 pesticides provided a more consistent effect in reducing the numbers of natural enemies per plant (Figure 6.2.26). During July, August and September the total number of natural enemies were lower on pesticide-treated plots than the control and fungicide-treated plots. As there was no effect of insecticide treatment on individual species of natural enemy it suggests that the insecticide treatment had a small effect on each, or several, natural enemies which was only observed once they were combined. The effectiveness during this trial could be a result of the shorter trial period where the insecticide would have remained effective. Insecticides could be a useful tool in predator exclusion experiments. Unfortunately, there was no effect of fungicide treatment on regulating the number of aphids infected by entomopathogenic fungi.

Comparison between field trial years

During 2011, the numbers of aphids and natural enemies recorded on plants were much lower than in 2010. When comparing the pattern of aphid numbers in the open

plot of the 2010 trial (6.1.4b) with the monitoring plots in 2011 (Figure 6.27a), the 2010 field trial appears to display three peaks (July, August and September) in aphid numbers, while the 2011 monitoring plots only display two (July and September). The occurrence of a third peak could be due to the crash occurring earlier during 2010, which would give the aphids more time to build up their population again in August. Furthermore, if emigration is the main factor contributing to an aphid population crash then this would also facilitate the faster population recovery during 2010, as the high numbers of emigrating aphids could recolonise the field trial plots.

Differences were observed in the most abundant type of natural enemy between 2010 and 2011, with 2010 seeing a relatively great presence of syrphid larvae and parasitoids, and 2011 seeing a relatively great presence of parasitoids and entomopathogenic fungi. Similar observations were made by Varenhorst and O'Neal (2012) who showed that *Harmonia axyridis* was the key predator of *A. glycines* in one year but that *Orius insidiosus* was more abundant in another year

As natural enemy communities appear to vary from year to year, with some years seeing a greater presence of one species compared to another, it seems likely that the aphid crash is a result of a community of natural enemies aggregating in response to high aphid numbers, rather than the specific effects of one or two natural enemies alone (Donaldson, *et al.*, 2007; Alyokhin, *et al.*, 2011). To confirm this effect for *N. ribisnigri*, it might be possible, as undertaken in other studies, to measure the effects of different individual predators by using partial exclusion methodology, where the use of different sized meshes would restrict different species of natural enemy and walls/raised cages would restrict or allow access to ground dwelling predators (Brosius, *et al.*, 2007b; Kidd and Jervis, 2007). This might indicate the most important community of complementary (no intraguild predation) generalist and specific predators, which one could then release into the field to see if the mid-summer crash could be manipulated to occur when required, which depending on the cost, could be implemented by growers. Alternatively, one could provide floral resources and mulches to increase the presence of these particular predators. For example, the provision of sweet alyssum has been shown to be successful in improving biological control by hoverflies and parasitic wasps in field-grown lettuce

(Johanowicz and Mitchell, 2000; Skirvin, *et al.*, 2011) and particularly for the control of *N. ribisnigri* (Gillespie, *et al.*, 2011).

Annual differences in the relative numbers of certain types of natural enemy could also be the result of intraguild predation, where natural enemies predate upon each other, therefore altering the presence and influence of members of the natural enemy community For example, the introduction of predators including Coccinellidae and Carabidae can reduce levels of parasitism of aphids by predation of mummies (Ferguson and Stiling, 1996; Snyder and Ives, 2001).

This study has evaluated the factors influencing the mid-summer crash of *N. ribisnigri* during 2010 and 2011. It is suggested that, of the factors discussed, natural enemies and emigration are the two key factors likely to be influencing aphid abundance and these are likely to be acting together to reduce aphid infestations. Future work is required to determine the level of influence that they both have.

Determining the causes of the mid-summer crash may allow researchers to predict when it is going occur, so that growers can allow nature to reduce aphid populations instead of applying unnecessary insecticides. On understanding the factors which cause the midsummer crash, researchers may also be able to create the conditions where natural control of aphid populations is facilitated. However, because agroecosystems undergo frequent disturbances, which can interrupt control by natural enemies this could make the creation of these environmental conditions challenging. This is also why the development of a control strategy for *N. ribisnigri* on lettuce using biological control agents continues to be challenging to implement (Landis, *et al.*, 2000).

Conclusion

- The mid-summer crash was observed during both trial years occurring between the 5-12 July 2010 and 14-29 July 2011.
- Netting treatments reduced the number of natural enemies in plots but did not completely exclude them. Netting treatments also influenced the microclimate and could have resulted in more aphids infected with entomopathogenic fungi during 2011.

- Fungicide and/or insecticide treatments did not reduce the numbers of natural enemies or aphids infected with entomopathogenic fungi during the 2010 trial. However, during 2011 the insecticide treatment was observed to reduce natural enemies in each trial month except June.
- Data on temperature and rainfall could not explain the decline in aphid numbers in 2010 or 2011. However, the intensity and duration of the wind and rain could not be excluded as a possible contributing factor.
- While alate numbers were low prior to the mid-summer crash, the increase in their percentage of the total population suggested emigration could be a significant contributing factor to the mid-summer crash, but further work is required to determine to what extent. During 2011 emigration resulting in poor plant quality was considered unlikely as the aphid decline still occurred in monitoring plots which contained lettuce plants of various ages.
- Natural enemies in both years increased prior to the mid-summer crash indicating they play a role in supressing aphid populations. Further work is required to determine the level of suppression natural enemies can achieve.
- Due to the variation in the species of natural enemies present each year, this study suggests that it is a community of natural enemies which contribute to the mid-summer crash rather than the activity of one or two key natural enemies.
- Emigration and natural enemies were the two main factors which could have influenced aphid populations in this study.

Chapter 7: Host Plant Resistance

Introduction

Host plant resistance is an alternative 'environmentally-friendly' method of control for certain pests and diseases, often providing absolute control. Originally the range and consequent use of resistant vegetable cultivars was limited but their value has increased following changes to pesticide regulations, continuing insecticide resistance issues and new technologies revolutionising plant breeding and GM approaches (Eigenbrode and Trumble, 1994). Resistant cultivars are now available for various 'vegetable' crops including potato, tomato, corn, brassicas and lettuce and can be used as part of IPM strategies to reduce insecticide applications and provide better control of pests overall. Particularly for a pest such as *N. ribisnigri*, which resides in the hearts of lettuce heads, resistant cultivars can provide a more effective control measure compared with foliar sprays of insecticide which may not reach the pest (Aarts, *et al.*, 1999).

Due to variations in genetics, plants can range from being completely resistant to susceptible to the damage caused by insect pests (Kogan, 1994). All plants exhibit some form of response to aphid feeding, although more 'resistant' plants have more effective antibiotic and/or antixenotic effects on insects (Kogan, 1994). Antixenosis resistance deters insects from colonising the plant, while antibiosis exerts an antibiotic/adverse effect on the pest, both of which can be due to plant structure (e.g. hard cell walls) and/or chemical composition (Caldwell, *et al.*, 2005). Plants can also express tolerance to insect pests by having the ability to withstand or repair damage inflicted by a pest, and this is also classed as a form of plant resistance (Reese, *et al.*, 1994). Crops such as lettuce, however, need to be pest-free and therefore the development of insect resistant cultivars with antixenosis and antibiosis is required. However, plants can express all three 'mechanisms of resistance' in their defence strategy (Gao, *et al.*, 2008).

In response to aphid feeding it is hypothesised that the different modes of resistance to aphid feeding are initiated through two processes. The first occurs in both susceptible and resistant plants where, in response to aphid feeding, changes in plant chemistry initiate a general stress response; while the second process involves

specific responses by plant resistance genes (R genes) on recognition of specific insect-induced elicitors (gene for gene interaction) (Smith and Boyko, 2007). The latter mechanisms are only present in resistant plants and the identification and exploitation of these R genes has led to the development of completely resistant cultivars providing pest-specific resistance.

Various insect R genes have been cloned, and structural similarities have been identified, such as leucine-rich-repeats (Kaloshian, 2004). This structural information is valuable as it can help to identify other R genes by screening for these repeats in germplasms. Currently, the largest number of R genes have been identified and characterized for the hessian fly, *Mayetiola destructor* (Anderson and Harris, 2006). Various R genes for aphids have been identified in cultivated vegetables including the *Mi*-1gene in tomato which confers resistance to the potato aphid, *M. euphorbiae* (Vos, *et al.*, 1998) and the *Vat* gene in melon which confers resistance to *A. gossypii* (Lombaert, *et al.*, 2009).

Cultivars resistant to *N. ribisnigri* are also available. The resistance was identified and developed over a period of 15 years and the first cultivar was released in 1999 (van der Arend, 2003). These resistant lettuce cultivars have since been widely cultivated throughout Europe (van der Arend, 2003). The resistance was originally sourced from the Institute for Horticultural Plant Breeding (IVT) *Lactuca* gene bank where some accessions of *Lactuca virosa* provided nearly complete resistance to *N. ribisnigri* (Eenink, *et al.*, 1982b). Inter-specific crosses were used to transfer this resistance into *L. sativa*. A bridging species *(Lactuca serriola)* was used, as offspring resulting from *L. sativa* and *L. virosa* crosses died (Eenink, *et al.*, 1982b). Breeding lines were supplied to private plant breeders to introgress the resistance into their own cultivars.

Since the development of these resistant host plants, several studies have attempted to understand the underlying resistance mechanisms. Investigation into the inheritance of the resistance in *L. sativa* confirmed that resistance is controlled by one dominant gene, known as the Nr-gene, with a 3:1 Mendelian segregation in F2 plants and with the potential existence of minor resistance genes (Eenink, *et al.*, 1982a). When making comparisons of several parameters (including mean relative growth rate, larval development/mortality, reproduction, honeydew production and

r_m) for WT *N. ribisnigri* on resistant and susceptible (near isogenic) lettuce lines, the absence of weight gain and honeydew production suggested that feeding is suppressed, if not absent, on the resistant line (van Helden, *et al.*, 1993). Other studies have also confirmed the effectiveness of the control of *N. ribisnigri* provided by these resistant lettuce cultivars (Liu and McCreight, 2006; Palumbo, 2002).

Studies on the effect of the Nr-gene on other species of lettuce aphid indicated complete susceptibility to *M. euphorbiae*, but partial resistance to *M. persicae.* However, reproduction of *M. persicae* varied significantly between the three homozygous resistant lettuce lines tested, suggesting that additional genes, interacting with the Nr-gene, are responsible and not the Nr-gene alone (Reinink and Dieleman, 1989). Therefore, the resistance mechanism associated with the Nr-gene is unlikely to be mechanical or due to a general change in plant quality, as it is likely that other aphid species would be affected in these circumstances, so the resistance is more species-specific (Tjallingii and Esch, 1993).

Advances in technology have since allowed researchers to associate the resistance mechanism with the phloem sieve elements using Electrical Penetration Graphs (EPG), where the near absence of the food uptake phase (E2 pattern) has been recorded on resistant lettuce lines (van Helden, 1993; van Helden and Tjallingii, 1993; ten Broeke, *et al.*, 2010). Further studies comparing the chemical composition of phloem sap from susceptible and resistant lines have shown that phloem samples collected from susceptible lines, by Ethylenediaminetetraacetic acid (EDTA) facilitated exudation, were preferred to phloem samples from resistant lines, indicating the presence of a feeding deterrent (van Helden, *et al.*, 1994; van Helden, *et al.*, 1995). The feeding deterrent could also be mobile, as the use of excised leaves results in a loss of resistance (Liu and McCreight, 2006); although this could also be due to the changes in metabolism resulting from the excision (Gao, *et al.*, 2008). It has also been confirmed that the deterrent is not toxic, as normal feeding and growth is resumed on transfer of aphids to susceptible lettuce cultivars (van Helden, *et al.*, 1993). Currently, the precise mechanism still remains unknown.

While the development of host plant resistant cultivars is an effective method of control against various pest insects, the possibility of selection for resistance-breaking biotypes has been considered, because of the increased selection pressure

provided by monocultures of resistant lettuce and the monogenic nature of the resistant genes (van der Arend, 2003). Furthermore, evidence for the occurrence of resistance-breaking biotypes has already been reported for other crops, such as *Amphorophora agathonica* which overcame the *Ag1* gene in raspberry (Dogimont, *et al.*, 2010). Therefore, it came as no surprise that, in 2007, a new biotype of *N. ribisnigri* was identified, which was able to develop and reproduce on resistant lettuce cultivars in Germany and France. Unfortunately, it was confirmed during 2009 that this new resistance-breaking biotype (Rb as assigned in this study) had reached the United Kingdom and samples were collected from a resistant lettuce crop in Kent and sent to Warwick Crop Centre.

Preliminary investigations into the new biotype(s), collected from Germany and France, on resistant and susceptible lettuce, confirmed that these aphids could develop on both types of cultivar, but the rate of population increase by resistance-breaking aphids was halved compared with the wild-type (WT) aphids on susceptible lettuce cultivars (Smilde, *et al.*, 2009). Use of the EPG technique showed that the Rb biotype expressed the same feeding characteristics when feeding on resistant cultivars as the WT biotype displayed when feeding on susceptible cultivars, indicating that Rb aphids are insensitive to the resistance mechanism affecting WT aphids (ten Broeke, *et al.*, 2010). How Rb aphids overcome the resistance provided by the Nr-gene remains unknown.

With the development of this new resistance-breaking *N. ribisnigri* biotype, the future effectiveness of the current Nr-gene cultivars in the control of this pest is uncertain, and the development of new resistant cultivars with new mechanisms of resistance is necessary. This study aims to identify sources of novel resistance to *N. ribisnigri* by screening lettuce cultivars and their wild relatives using WT and Rb *N. ribisnigri*. Successful plants will be sources of genetic material for the development of new resistant cultivars.

Methods

7.1 Preliminary screen to confirm resistance

Six Butterhead cultivars were obtained from different breeding companies and screened with either WT (4850a) or Rb (RbKentPop) *N. ribisnigri* to give 12 treatments in total. Susceptible cultivars, Clarion and Charles, were sourced from Enza zaden and Nunhems respectively, and four resistant cultivars (Nr-gene), Aljeva, Malfalda, Skyphos and Rotary were obtained from Enza zaden, Nunhems, Rijk Zwann and Elsoms respectively.

The Butterhead cultivars were sown in vermiculite on 5 April 2010. On 12 April, ten seedlings of each cultivar were transferred into individual pots and placed in CE Room 4 at 18°C 16L:8D. The plants were used two weeks later when their 4th true leaf had unfolded (BBCH growth stage 14).

New born nymphs of WT and Rb *N. ribisnigri* were obtained by releasing 180 alates of each biotype into separate cages containing six lettuce plants (cv. Saladin). These were left for 24 hours to produce nymphs of similar age.

After 24 hours, eight new born WT nymphs were inoculated onto five plants of each Butterhead cultivar and eight new born Rb nymphs were inoculated onto the remaining plants. The plants were then covered with micro-perforated polypropylene bags (200mm x 500mm; Cryovac®), which were secured with an elastic band and kept at 20°C 16L:8D in CE Room 6. The plants were left for nine days, after which the numbers of *N. ribisnigri* surviving were recorded. Plants were arranged on a single shelf in a randomised row and column design (4 x15 plants) with 12 treatments, five blocks and 60 plants in total.

7.2 Screening a lettuce genetic diversity set with WT and Rb *N. ribisnigri* for new sources of resistance

A lettuce diversity set consisting of 96 lines (see Appendix Table A2) was obtained from the Vegetable Genetic Improvement Network (VeGIN) at The Warwick Crop Centre at The University of Warwick. The lettuce diversity set represents a range of

morphological and genetic variation in domesticated *L. sativa* accessions and wild *Lactuca* species. Table 7.2.1 displays the types and numbers of lines comprising the set and Figure 7.2.2 illustrates this variation.

Table 7.2.1 Crop types and numbers of lines comprising the genetic diversity set.

Crop Type	Number
Butterhead	25
Cos	17
Wild	17
Crisp	10
Cutting	10
Latin	7
Batavian	4
Stem	2
Iceberg	1
Leaf	1
Oilseed	1
Stalk	1

Figure 7.2.2 The 96 lines comprising the lettuce diversity set.

For each of the 96 lines, four seeds were sown together in a single pot and kept in CE Room 4 (18°C 16L:8D). After two weeks, the seedlings were transplanted into individual pots. Depending on the germination success of the seeds, 1-4 plants were available for each lettuce line. This process was repeated five times on 1 February, 9 March, 4 April, 4 May and 16 June 2011. Any lines identified as being poor at germinating after the initial sowing occasion had extra seed sown in vermiculite thereafter.

The screening experiments were repeated ten times and each replication of the WT *N. ribisnigri* screening experiment was designed to screen all the 96 lines with WT (4850a biotype) *N. ribisnigri.* However, due to poor germination, this was not always achievable and some screens would have several lines missing. As a result tests on each lettuce line were replicated between four and ten times depending on germination success. The lines used for the WT *N. ribisnigri* screen were arranged in an alpha design (leaving spaces for any lettuce lines which did not germinate).

When there were too few remaining plants to carry out a further screen with WT *N. ribisnigri*, they were used to screen Rb *N. ribisnigri* (RbKent biotype). Such plants were used from sowing occasions on 4 April, 4 May and 16 June. Further seeds were sown on 29 July 2011 to screen further Rb *N. ribisnigri*. The screening experiment for the Rb *N. ribisnigri* was repeated five times and each line had one to seven replicates. A list randomiser program was used to create a randomised design for the Rb *N. ribisnigri* screen (Haahr, 1998).

The lines which germinated successfully for the WT *N. ribisnigri* screen were used between 23 days (4^{th} - 5^{th} true leaf unfolded depending on the line) and 42 days old (when grown at 18°C 16L:8D), as the replications were staggered to make data collection manageable. Lines used to screen Rb *N. ribisnigri* were used between 34 and 43 days day old.

New born nymphs were used in the experiments; these were obtained by inoculating 15 WT or Rb *N. ribisnigri* alate and apterous adults per 20 plants (cv. Pinokio). These were then left for 48 hours at 20°C 16L:8D in CE Room 3 to produce 1-2 day old nymphs. Five nymphs were then transferred to each lettuce line using a fine paint

brush. The inoculated plants were covered individually with micro-perforated polypropylene bags (200mm x 500mm; Cryovac®) which were secured with an elastic band and left for three weeks. Both screens were arranged on a single shelf in CE Rooms 3 or 6, at 20°C 16L:8D.

After approximately 3-4 weeks (ranging from 20 to 29 days for WT *N. ribisnigri* and 21-26 days for Rb *N. ribisnigri* - varying due to the time required to count the aphids), the aphids on each lettuce line were counted, recording the number of alate and non-alate (apterous and nymphs) *N. ribisnigri* present. Data collection was carried out over a two day period.

7.3 Screening mapping parents with WT and Rb *N. ribisnigri* for new sources of resistance

An additional screen was carried out to investigate the phenotype for resistance to WT and Rb *N. ribisnigri* in six mapping parent lines, compared with two susceptible lines. Some of these lines (2, 20, 87, 94 and 96) had been screened previously in Experiment 7.2 but had low replication numbers. Saladin (Line 1) was also included as it was absent in Experiment 7.2. Other lines were re-screened because they had shown good resistance levels previously. The additional lines included a parent of a Saladin cv. TILLING (Targeted Local Lesions IN Genomes) population (LJ09003), and a new Salinas mapping parent line (LJ10221). Screening these eight lines would allow more informed decisions about the mapping parents that should be used in future crosses, through knowing their phenotype for resistance to WT and Rb *N. ribisnigri.*

A screen of these eight lines with Rb and WT *N. ribisnigri* resulted in 16 treatments. Originally the design was created for five replications per treatment (80 plants in total) but poor germination of lines 16, 18 and 19 meant that only four replications were carried out (64 plants in total). These screens were carried out as per Experiment 7.2 and were repeated twice (20 and 21 December 2011).

Twenty seeds were sown per line as described in Experiment 7.2 and kept in CE Room 4 (18°C 16L:8D). Plants were used when their 4^{th} - 5^{th} true leaf had unfolded which varied between lines. Both screens were carried out in CE Room 6 (20°C

16L8D). Each replication was arranged on a single shelf in a randomised row and column design (4 x 16). Five new born nymphs were inoculated per plant and were obtained as per Experiment 7.2. Treatments were left for three weeks (21 and 22 days), and the aphids on each lettuce line were counted, recording the number of alate and non-alate (apterous and nymphs) *N. ribisnigri* present. Data collection was carried out during one day.

7.4 Screening wild relatives of lettuce with Rb *N. ribisnigri* for new sources of resistance

A selection of wild relatives of lettuce, including 14 *Cichorium intybus* and 18 *Cichorium endivia* accessions (see Appendix Table A3), were screened using Rb *N. ribisnigri*. Seeds were sown on 10 November 2011 in a glasshouse, and plants were transferred to CE Room 3 (20° 16L:8D) on 16 December 2012 and left to acclimatise whilst arranged in an alpha design on a single shelf.

On 18 December 2011, the plants (were each inoculated with five new born Rb *N. ribisinigri* nymphs. The new born nymphs were obtained as per Experiment 7.2. Plants were 38 days old when screening began which was at the growth stage when their 4^{th} - 5^{th} true leaf had unfolded (which varied between lines).

A total of 32 accessions were screened and four replicates of each accession were tested at the same time. Data collection was carried out over a period of two days on 9 and 10 January 2012, 22 days from inoculation. The numbers of alate and non-alate (apterous and nymphs) were recorded.

Results

7.1 Preliminary screen to confirm resistance

Analyses of the mean percentage of WT and Rb *N. ribisnigri* surviving (out of eight inoculated) per plant on Day 9 were performed using ANOVA. An angular transformation was carried out on the percentages to normalise the data. Due to the zero values present in the data set, the ANOVA was carried out including the zero values, and also excluding the zero values by removing them from the data set.

Figure 7.1.1 shows the back-transformed means of the proportion of *N. ribisnigri* surviving per plant after 9 days on each of the treatments. The WT *N. ribisnigri* biotype survived on both susceptible cvs. Clarion and Charles but could not survive on the resistant cvs. Aljeva, Skyphos and Rotary. Only one WT aphid was still alive on Malfalda.

The WT biotype on cv. Clarion and the Rb biotype on cv. Skyphos equally had the highest proportions of aphids surviving per plant after nine days, each with a proportion of 0.975 aphids surviving per plant. Out of all of the treatments with high survival, the Rb *N. ribisnigri* biotype on cv. Malfalda had the lowest number of surviving aphids. Rb *N. ribisnigri* survived on both resistant and susceptible cultivars.

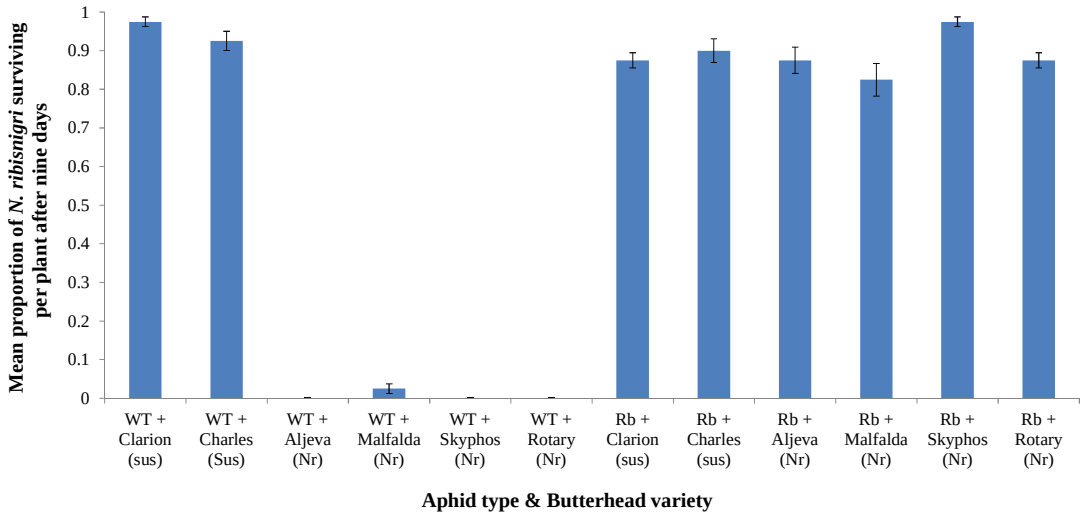


Figure 7.1.1 Back-transformed mean proportion of WT and Rb *N. ribisnigri* biotypes surviving per plant on two susceptible (cvs. Clarion and Charles) and four resistant Butterhead (cvs. Aljeva, Malfalda, Skyphos and Rotary) cultivars after nine days.

Table 7.1.2 shows the treatment means and LSD values used to determine significance between the treatments when the analysis included the zero values. Treatment had a significant effect on the mean percentage of aphids surviving per plant on Day 9 (F (11,48)=54.06, p<0.001). The numbers of aphids of the WT *N. ribisnigri* biotype on the two susceptible cvs. Clarion and Charles were significantly different to the numbers of aphids of the WT biotype on the resistant cultivars.

The WT biotype on cvs. Charles and Clarion performed similarly to all the Rb treatments, with the exception that the WT biotype on cv. Clarion was significantly different to the Rb biotype on cv. Malfalda. All the treatments with high numbers of aphids surviving at Day 9 were significantly different from the WT *N. ribisnigri* biotype on cvs. Aljeva, Malfalda, Skyphos and Rotary, where 100% mortality was observed and expected.

Table 7.1.2 Angular transformed treatment means of the percentage of WT and Rb *N. ribisnigri* surviving per plant on Day 9, degrees of freedom and 5% LSD from the ANOVA analysis including the zero values. Means with different letters are significantly different.

Treatment	Mean	d.f.	LSD
WT + Clarion	85.9^{a}	*48*	*14.64*
WT + Charles	79.9^{ac}		
WT + Aljeva	0^{b}		
WT + Malfalda	4.1^{b}		
WT + Skyphos	0^{b}		
WT + Rotary	0^{b}		
Rb + Clarion	71.6^{ac}		
Rb + Charles	78.0^{ac}		
Rb + Aljeva	74.2^{ac}		
Rb + Malfalda	70.8^{c}		
Rb + Skyphos	85.9^{a}		
Rb + Rotary	71.6^{ac}		

Table 7.1.3 shows the treatment means and LSD values (two values are present for the comparison of treatments with different numbers of replicates) used to determine significance between the treatments means which had a value above zero. Therefore, treatments with WT aphids on cvs. Aljeva, Skyphos and Rotary were excluded. Treatment had a significant effect on the percentage of aphids surviving per plant on Day 9 (F (8,32)=2.97, p<0.013). This analysis showed all the treatments performed similarly and were only significantly different to the WT biotype on cv. Malfalda,

where 100% mortality was expected. Little difference was observed between this analysis and the previous analysis which included the zero values.

Table 7.1.3 Angular transformed treatment means of the percentage of WT and Rb *N. ribisnigri* surviving per plant on Day 9, numbers of replicates, degrees of freedom and 5% LSD for the ANOVA analysis, excluding the zero values. Means with different letters are significantly different.

Treatment	Mean	Rep	d.f.	LSD
WT + Clarion	85.9[a]	*5*	*32*	*17.66 Max-max rep*
WT + Charles	79.9[a]	*5*		*30.60 Max-min rep*
WT + Aljeva				
WT + Malfalda	4.1[b]	*1*		
WT + Skyphos				
WT + Rotary				
Rb + Clarion	71.6[a]	*5*		
Rb + Charles	78.0[a]	*5*		
Rb + Aljeva	74.2[a]	*5*		
Rb + Malfalda	70.8[a]	*5*		
Rb + Skyphos	85.9[a]	*5*		
Rb + Rotary	71.6[a]	*5*		

7.2 Screening a lettuce genetic diversity set with WT and Rb *N. ribisnigri* for new sources of resistance

The mean number of aphids counted per plant from Experiment 7.2 on the WT and Rb *N. ribisnigri* biotypes (alate and all stages) were analysed using a Restricted Maximum Likelihood Analysis (REML). The WT and Rb *N. ribisnigri* data were analysed separately as the experiments were designed independently**.** A LOG10 transformation was carried out to normalise the count data and a value of one was added to the data due to presence of zero values within the replicates. Data interpretations were made using the predicted mean and 5% LSD values. While the

REML output provided individual LSDs for all comparisons between all of the 96 lines, alternative LSDs were calculated to simplify the analysis of the data. A single LSD was calculated for the numbers of all stages and alates by multiplying the t value (calculated from the probability and degrees of freedom) by the maximum standard error of the difference.

In the analysis of the number of WT *N. ribisnigri*, the plant lines were used as the fixed model to determine whether they had an effect on the numbers of aphids recorded. In addition, a random model was used to determine the effects of replicate, block and plot according to the alpha design.

The output of the REML showed that there was little effect of replicate, block and plot on either the number of all stages or alates of *N. ribisnigri* recorded on each line. There was a statistically significant effect of line on the numbers of all stages recorded (ndf 95, ddf 647, $p<0.001$) and on the numbers of alates (ndf 95, ddf 647, $p<0.001$) for the WT *N. ribisnigri* biotype. The numerator degrees of freedom (ndf) represents the variance due to the effect and the denominator degrees of freedom (ddf) represents the variance within treatments (Weinberg and Abramowitz, 2008).

Figure 7.2.3 shows the predicted transformed means calculated for all stages and alates of WT *N. ribisnigri* per plant on each line screened. Line 95 was the most resistant line and had the lowest number of all stages and alate aphids (0.25 and 0.05 per plant respectively). These means were significantly different from the means for all other lines including Line 1 (Saladin) and Line 2 (Iceberg), which are commercial cultivars. Line 65 was the most susceptible line with the highest number of all stages and alate aphids per plant (1.83 and 2.49 respectively) and was significantly more susceptible to *N. ribisnigri* than Lines 1 and 2.

When analysing the data on Rb *N. ribisnigri*, the lettuce lines were included as the fixed model to determine whether they had an effect on the numbers of aphids recorded (total number and number of alates). In addition, a random model was used, which showed the effects of replication.

There was little effect of replication on the numbers of aphids recorded per plant (all stages and alates). However, there was a statistically significant effect of plant line

on the number of all stages recorded (ndf 90, ddf 224, $p<0.001$) and on the number of alates (ndf 90, ddf 224, $p<0.001$).

Figure 7.2.4 shows the predicted means estimated for the numbers of all stages and alates of Rb *N. ribisnigri* per plant on each line. Line 95 was the most resistant line and had the lowest total number of all stages and alates per plant (0.02 and 0.21 per plant respectively). Line 85 was the second most resistant line.

When using the LSDs to compare means, the numbers of all stages on Line 95 were significantly different from Line 93 and upwards, and numbers of alates were significantly different from line 96 and upwards. When compared to Line 2 (Line 1 not screened), Line 95 was significantly more resistant. Line 52 was the most susceptible line with the highest numbers of all stages and alate aphids per plant (2.12 and 2.87 per plant respectively). When compared with Line 2, Line 52 was significantly more susceptible when considering both the total number of aphids and the number of alates.

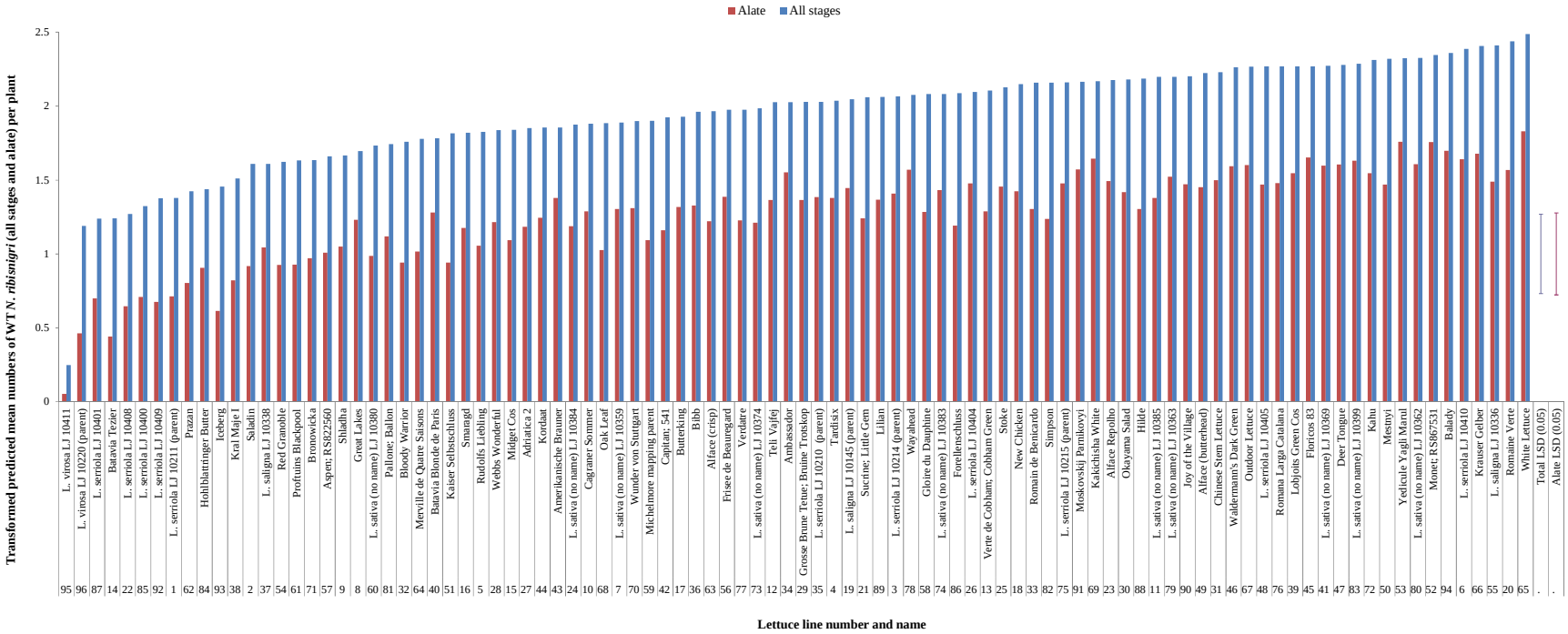


Figure 7.2.3 Transformed predicted mean numbers of WT *N. ribisnigri* per plant (all stages and alate) from the REML analysis.

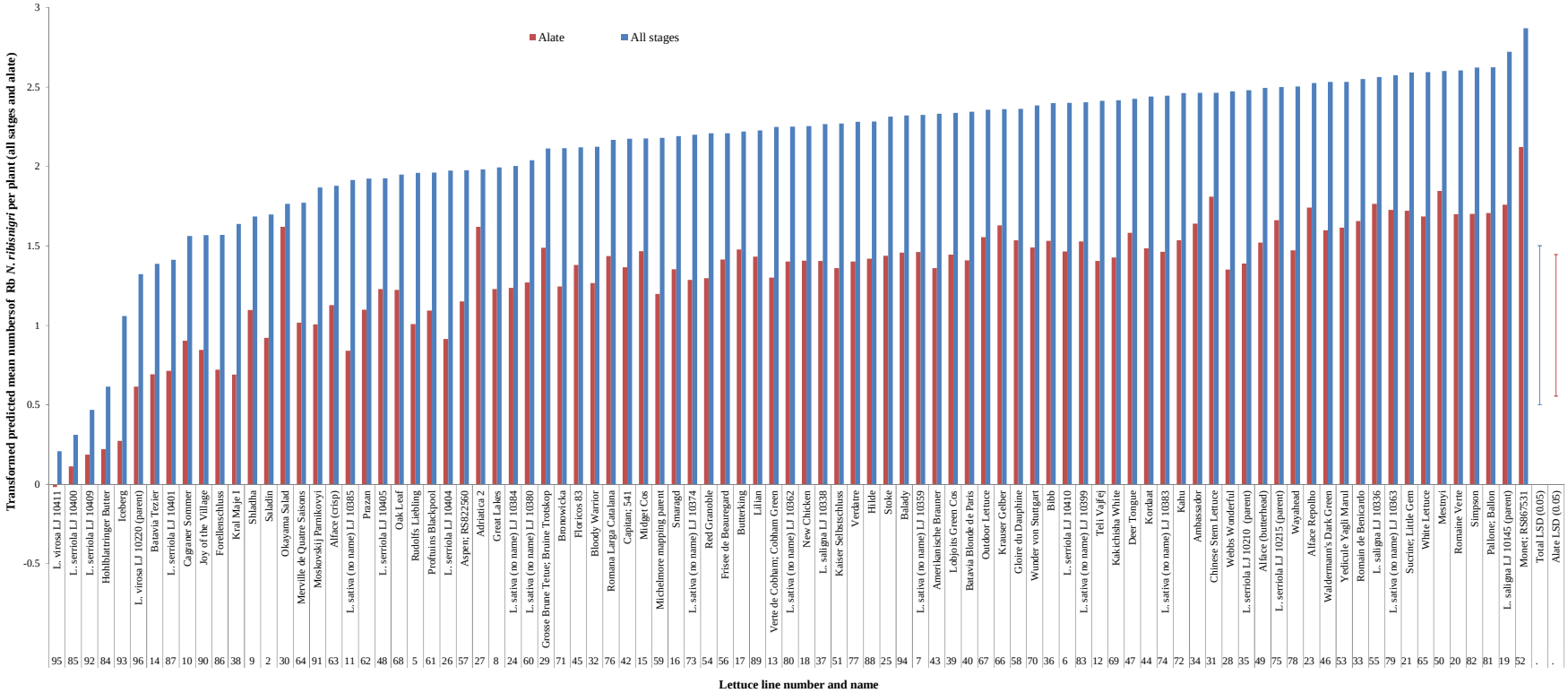


Figure 7.2.4 Transformed predicted mean numbers of Rb *N. ribisnigri* per plant (all stages and alate) from the REML analysis.

7.3 Screening mapping parents with WT and Rb *N. ribisnigri* for new sources of resistance

Analyses of the number of *N. ribisnigri* (alate and all stages) recorded per plant on the mapping parents were performed using ANOVA, following a LOG10 transformation to normalise the data. An ANOVA was performed separately for the number of alates and all stages. A value of one was added to all the data as some values were zero and could not be transformed. Interpretations of the data were made using treatment means and 5% LSD values.

The ANOVA showed a significant effect for plant line for alates ($F(7,102)=6.21$, $p<0.001$) and all stages of *N. ribisnigri* ($F(7,102)=8.43$, $p<0.001$). There was also an interaction between plant line and aphid biotype for alates ($F(7,102)=2.67$, $p<0.014$) and all stages ($F(7,102)=2.27$, $p<0.035$).

Figure 7.3.1 shows transformed means for both the number of all stages and alates per plant of WT and Rb *N. ribisnigri* recorded on five mapping parents (LJ09001, LJ09002, LJ10220 and LJ10222), one TILLING line (LJ09003) and two susceptible lines (LJ10336 and LJ10410). LSD (0.05) values are shown to allow comparisons between transformed means.

LJ10220 was the most resistant line for both alates and all stages of the Rb biotype with 0.67 and 1.38 mean aphids per plant respectively. Line LJ09003 was the most resistant line for both alates and all stages of the WT biotype with 0.966 and 1.582 mean aphids per plant respectively. However, no significant difference in performance was observed when the best performing treatments for Rb and WT *N. ribisnigri* were compared.

When using the LSD to compare means, performance of the WT biotype on the two susceptible lines was significantly higher when compared to the line which was most resistant (LJ09003) and also LJ09001, LJ09002, LJ0220 and LJ10221. This was also observed for the Rb biotype on its most resistant line (LJ10220) when compared to both the susceptible lines. The performance of the Rb biotype on the susceptible line LJ10336 was also significantly different to its performance on LJ9002 and LJ9003. The two aphid biotypes performed similarly on each line except for line LJ10221

where the Rb biotype produced significantly more aphids in total than the WT biotype.

Significantly more alate aphids were produced by Rb biotypes on lines LJ09003 and LJ10221 compared with the WT biotype. Numbers of alates of both WT and Rb *N. ribisnigri* were similar on the two susceptible lines. The WT biotype had significantly less alates on line LJ09001, LJ09002, LJ09003, LJ10220 and LJ10221 compared to the susceptible line LJ10336 and there were significantly less alates on line LJ09003 compared with susceptible line LJ10410. The Rb biotype had significantly less alates on line LJ10220 when compared to both the susceptible lines.

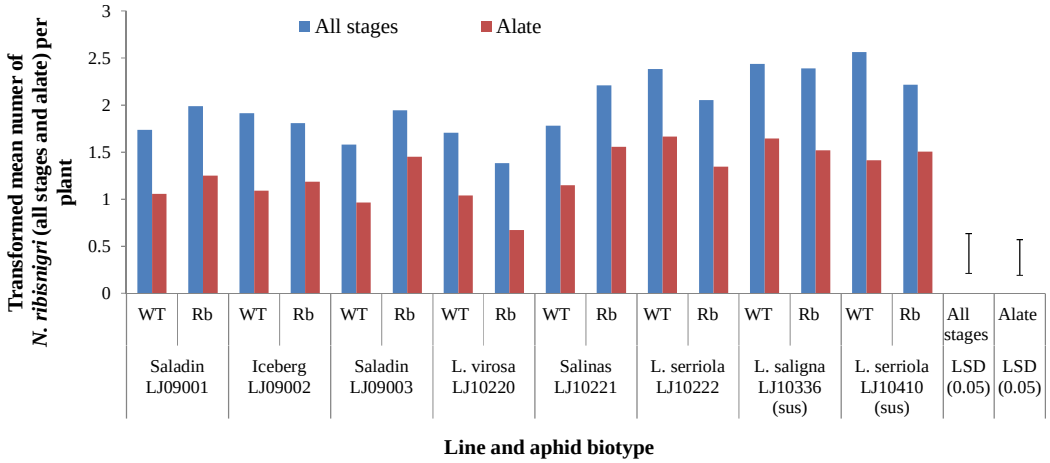


Figure 7.3.1 Transformed mean numbers of WT and Rb *N. ribisnigri* biotypes (all stages and alates) recorded per plant after three weeks on lettuce lines consisting of five mapping parents, one parent of a TILLING line and two susceptible (sus) cultivars. LSDs (5%) are shown for all stages and alates.

7.4 Screening wild relatives of lettuce with Rb *N. ribisnigri* for new sources of resistance

Analyses on all stages and alates of the Rb *N. ribisnigri* biotype were performed using a REML analysis. The data were normalised using a LOG10 transformation and a value of one was added to all data due to the presence of zeros in the data set. The fixed model included the lines screened, and the random terms which could affect the fixed model included replication, row and column. Interpretations of the data were made using the F statistic, predicted treatment means and 5% LSDs provided by the REML analysis. Individual LSDs are provided in Figure 7.4.1 for the number of all stages and number of alate *N. ribisnigri,* which was calculated by multiplying the t value (calculated from the probability and degrees of freedom) by the maximum standard error of the difference. This was done because the large number of LSDs provided from the REML output could not be displayed.

The REML suggested there was only a small amount of variation due to the effect of the random terms (replication, row or column) on the total numbers and numbers of alate Rb *N. ribisnigri* recorded, suggesting that there was no effect of plant position in the design. A significant effect between the lines was found when analysing both the number of all stages of *N. ribisnigri* (ndf 31, ddf 75, $p<0.001$) and the number of alates (ndf 31, ddf 76, $p=0.005$).

When analysing the transformed predicted means in Figure 7.4.1, adjusted for the effects caused by the random terms (for all stages of *N. ribisnigri* recorded), the most resistant and least resistant lines were *C. intybus* species Lines 50 and 10 with means of 0.41 and 2.82 per plant respectively. Using differences between predicted means and LSD values for all stages of *N. ribisnigri* showed that Line 50 was significantly different to all other treatments, including Line 37, which was the next most resistant line with a predicted mean of 1.63 per plant. Line 13 was the first line which was significantly different from Line 37 in ascending order.

When analysing the predicted means for alate *N. ribisnigri,* Line 50 had the least alates and Line 10 had the most, with means of 0.24 and 1.61 per plant respectively. Both were *C. intybus* species. When looking at the LSD values, Line 26 with a

predicted mean of 1.17 per plant was the first line which was significantly different from Line 50 in ascending order.

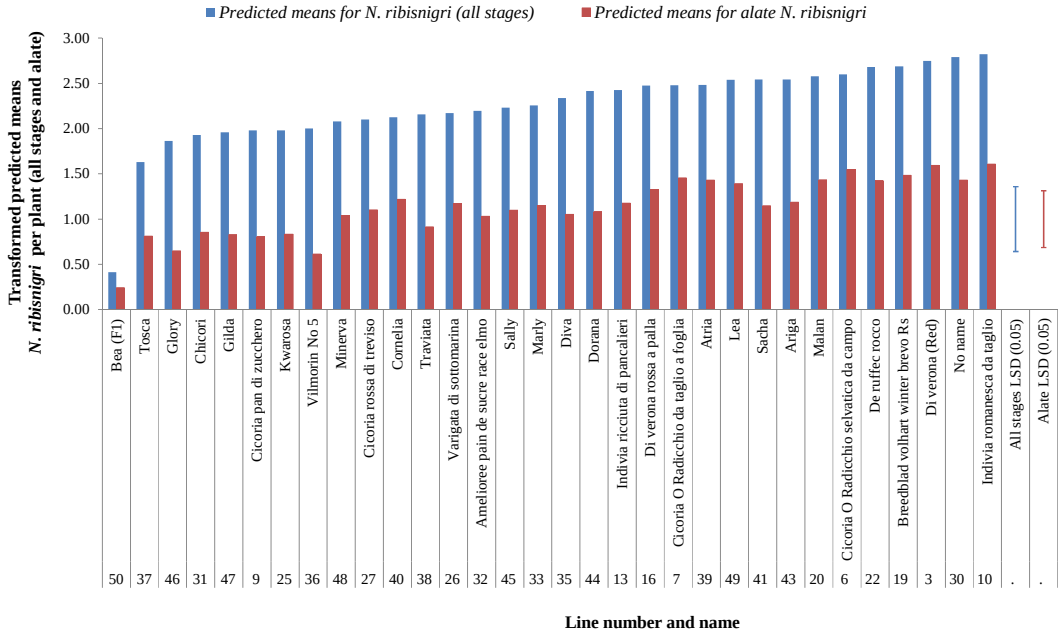


Figure 7.4.1 Transformed predicted means from a REML analysis of Rb *N. ribisnigri* (all stages and alate) per plant on each line screened. LSDs (5%) are shown for the numbers of alates and all stages.

Discussion

Experiment 7.1 confirmed the host plant-resistance-breaking phenotype of the Rb biotype collected from Kent as it survived on the resistant cultivars whilst the WT biotype did not. The study also suggested that there is no fitness trade-off acting against development and reproduction as a result of possessing the host plant resistance-breaking phenotype, as survival of Rb aphids on the resistant cultivars was comparable to the WT biotype on the control (susceptible) plants. However, other trade-offs might exist, as discussed in Chapter 3.

A large variation in the level of aphid infestation was observed between the 96 lines for both WT and Rb *N. ribisnigri.* This can be explained by the presence of resistant, partially resistant and susceptible lines, such as those observed when screening other crop germplasms (McCreight, 2008; Pelletier, *et al.*, 2010; Babura and Mustapha,

2012). Partial resistance in *L. virosa* is controlled by a recessive allele at the same locus as the (incomplete) dominant allele governing complete resistance (Eenink and Dieleman, 1983). It is thought that minor resistance genes could also exist and would explain the variations observed between a susceptible group of F2 *L. sativa* plants (Eenink, *et al.*, 1982a). Even different *L. virosa* accessions providing complete resistance have been reported to vary in the level of resistance they provide. Again, minor resistance genes influencing the Nr-gene could explain this, but also the existence of different Nr alleles has yet to be confirmed (Eenink and Dieleman, 1983). Variation between the lines could also be due to the age of the plant or foliage at the feeding site, which have been identified as factors influencing host plant resistance (Eenink and Dieleman, 1980; Kaloshian, *et al.*, 1995; Le Roux, *et al.*, 2008).

When screening the diversity set, Line 95, *L. virosa* (LJ 10411), was the most resistant line for both WT and Rb *N. ribisnigri* and is the original wild donor species of the Nr-gene used to develop current resistant cultivars. As the line also provides resistance to the Rb aphids it indicates that *L. virosa* (LJ 10411) must contain 'novel' resistance genes determining a different type of resistance mechanism to the Nr-gene in the host plant. The existence of other novel resistance genes in *L. virosa* is also supported by EPG recordings where both WT and Rb biotypes displayed strong reductions in phloem sap ingestion when feeding (ten Broeke, *et al.*, 2010).

Line 96 was the second most resistant line to WT *N. ribisnigri*, but other lines provided enhanced resistance to the Rb biotype. For example, Lines 84, 85 and 92 were more resistant than Line 96 and appeared to show greater resistance to Rb *N. ribisnigri* than to the WT biotype. While the data for Lines 84, 85 and 92 were based on a low number of replicates, this could suggest that the genetically-determined mechanism of resistance in these lines which may be either, or a mixture of, antibiosis and antixenosis, is more effective against the Rb biotype, and therefore not the same mechanism as the Nr-gene.

Evaluation of different *N. ribisnigri* Rb populations through EPG recordings has shown variation in the phase associated with food uptake, with some populations feeding more easily than others on a resistant line (ten Broeke, *et al.*, 2011). This suggests that there may be genetic variation in the response of aphids to the same

plant species and future work should evaluate different aphid populations of both WT and Rb biotypes.

The majority of the lines which displayed superior resistance to WT and Rb *N. ribisnigri* were wild species, indicating that they are probably the most important source of useful resistance traits. A dendrogram was created to summarise the genetic relationships between the 96 accessions (lines) (Figure 7.5). The lines were separated by morphological grouping into two clusters which separated the wild species (Cluster 1) from the domesticated cultivars (Cluster 2), illustrating the distinct genetic differences between the two groups (Walley, *et al.*, In preperation). Within Cluster 2, there was further separation into three sub-clusters, where Cluster 2a formed a clade that was distinct from Clusters 2b and 2c. This indicated that Cluster 2a was more closely related to the wild species in Cluster 1 than Cluster 2b and 2c were, which suggests that it contains species that were domesticated at an early stage from the wild species. Clusters 2b and c are more distantly related to the wild species and represent the more domesticated species. Analysis of the genetic relationships of the domesticated cultivars resulted in Butterhead and Cos crop types being grouped into Cluster 2b and 2c respectively.

When considering the resistance levels of each of these clusters to WT *N. ribisnigri,* the groupings support the hypothesis that wild species contain more genetic variation, and therefore a wider range of phenotypes resistant to WT *N. ribisnigri,* than more domesticated cultivars. An overall mean for the numbers of all stages of WT *N. ribisnigri* per plant was calculated for the lines within each cluster. Cluster 1 was the most resistant cluster with the lowest mean number of WT *N. ribisnigri* per plant (120.73 aphids per plant), and this represented the wild species. This was followed by Cluster 2a (125.4 aphids per plant), which was most closely related to the wild species and therefore likely to have more genetic variation and potential resistance compared with Cluster 2b (145.15 aphids per plant) and Cluster 2c (169.90 aphids per plant). A similar pattern was observed using overall means for the Rb biotype, where Cluster 1 was the most resistant (105.21 aphids per plant) followed again by Cluster 2a (160.38 aphids per plant), Cluster 2b (210.94 aphids per plant) and Cluster 2c (268.61 aphids per plant). This suggests that early in the domestication process, traits such as improved taste, delayed bolting, increased seed

size, head appearance and non-shattering seeds (de Vries, 1997) were selected preferentially, which may have resulted in the loss of genetic diversity and variation for other beneficial traits such as resistance to *N. ribisnigri*. This emphasises the importance of wild species and their relatives in providing new sources of genetic variability for beneficial traits such as resistance to insects (el Bouhssini and Nachit, 2000; McCreight and Liu, 2012). Similarly, the screen of *C. intybus* and *C. endive* accessions in the present study identified a line (Line 50) with superior resistance to Rb *N. ribisnigri*.

Unfortunately, breeding traits identified in wild species and distant relatives into commercial cultivars can be challenging, with their genetic compatibility determining the viability and length of the breeding process (Dogimont, *et al.*, 2010). For example, crossing *L. virosa* and commercial lettuce cv. *L. sativa* results in non-viable offspring, even though they belong to the same genus, *Scariola,* in the *seriolla* group (Lindqvist, 1960). As a result, a bridging species *L. serriola* (which is the closest relative of *L. sativa)* was required (Eenink, *et al.*, 1982b). This incompatibility can be explained by chromosome morphology, since *L. virosa* is very different to *L. sativa*, whilst *L. sativa* and *L. serriola* are very similar and more closely related (Lindqvist, 1960). Therefore, useful traits associated with candidate genes from other species, such as *L. serriola*, would be preferred, as it would remove the need for a bridging species and reduce the time required to produce new cultivars. Unfortunately, in this study, the lines expressing high resistance were both *L. virosa* species. Other researchers have since been successful in identifying an accession of *L. serriola* which is resistant to WT *N. ribisnigri* (McCreight and Liu, 2012).

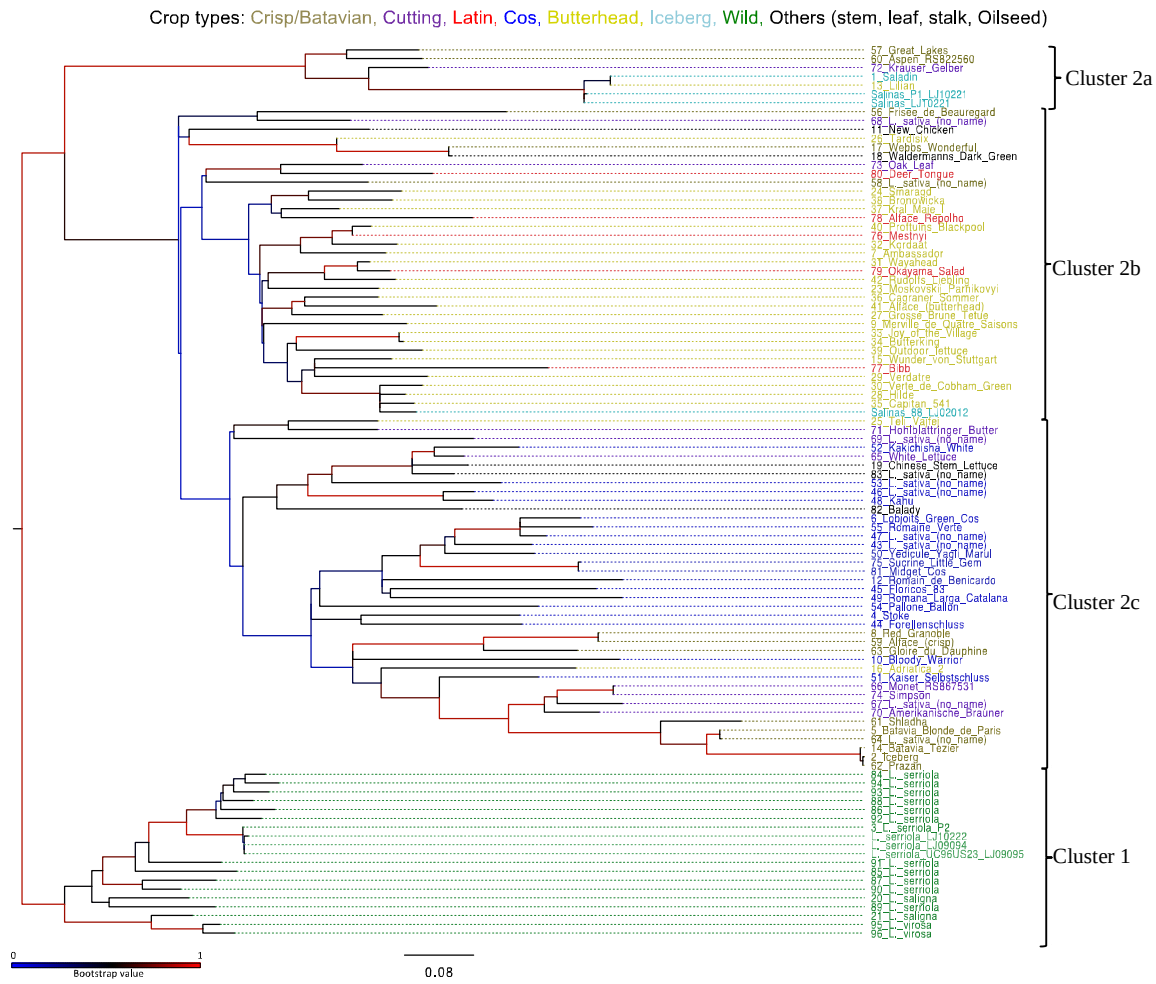


Figure 7.5 Genetic relationships in the VeGIN lettuce diversity set. The bootstrap value indicates the level of confidence in the relationships which is determined by assessing the repeatability of the results via a method known as bootstrap (Felsenstein, 1985). Red lines represent relationships where there is a high level of confidence in their accuracy.

Research has shown that resistance to insects in plants can be monogenic, but more usually, resistance is a quantitative trait, which varies in degree due to the action of two or more genes and is responsible for controlling partial resistance (Yencho, *et al.*, 2000). The Nr-gene is an example of monogenic resistance which following introgression into *L. sativa* is inherited dominantly, so that plants show complete resistance. In contrast, plants expressing partial resistance have continuously-varying levels of resistance which are controlled by one or more genes (polygenic) (Singh

and Singh, 2005). A comprehensive review of the monogenic and polygenic genes involved in aphid resistance in a range of crops has recently been generated by Dogimont *et al.* (2010).

With the development of molecular tools, scientists are increasingly able to identify the regions of DNA containing genes involved in quantitative traits (polygenic genes), which are designated as quantitative trait loci (QTL). For example, recent work on *P. persicae* has shown that the QTLs involved in resistance to *M. persicae* co-locate with QTLs involved in feeding behaviour (Sauge, *et al.*, 2012). While in soybean, two QTLs associated with resistance to *A. glycines* are linked to high isoflavone content, resulting in antibiosis resistance (Meng, *et al.*, 2011). These studies indicate that the mechanisms of host plant resistance to aphid species could vary greatly.

Mapping of QTL regions and other resistance genes requires a mapping population for that species. This is a population created from crossing two parent plants that have highly contrasting phenotypes for the trait being investigated, and also have DNA polymorphisms which can be used as molecular markers. The segregation patterns of the molecular markers in the offspring can then be analysed by genotyping the individual lines. Mapping software can be used to place the molecular markers in order, indicating relative genetic distances between the markers (Prasanna, 2007). The approximate location of the gene controlling the trait can then be determined, as any molecular markers which are continually inherited with the trait are likely to be closely located to the gene/genes controlling that trait (Lodge, *et al.*, 2007). Therefore, accessions with associated mapping populations have valuable genetic information which can be used for mapping genes involved in resistance.

Due to the convenience of working with candidate plants which already have mapping populations available, an additional screen of selected mapping parents was carried out in this study. This included re-screening some of the 96 lines but also included a Salanis (LJ10221) mapping parent and a Saladin TILLING population parent. While none of the lines provided complete resistance to Rb and WT *N. ribisnigri*, Line 96 was the most resistant line against Rb *N. ribisnigri*, and the TILLING parent was the most resistant line against WT *N. ribisnigri*, although not

significantly different from Line 96. The screen also confirmed previous observations, that Salanis was susceptible to *N. ribisnigri* (McCreight, 2008).

The present study has identified potential sources of resistance to Rb and WT *N. ribisnigri*, which could be investigated further. It also emphasises the importance of maintaining germplasm, as access to the genetic variation in wild species is crucial for discovering novel resistance. With the development of molecular technology, research should focus on introducing polygenic resistance, which would be more resilient, as it would be more challenging for the aphids to overcome. This is in contrast to monogenic resistance, where the development of new aphid biotypes has already been observed (Dreyer and Campbell, 1987). Furthermore, as TILLING populations are often used to create genetic variation in domestic crops, particularly where much of the wild variation has been lost (Slade and Knauf, 2005), it would be useful to screen the mutagenised seeds of this parent to see if any mutations resulted in superior resistance to WT or Rb *N. ribisnigri*.

Conclusion

- The Rb biotype collected from Kent survived on Nr-gene cultivars of lettuce and the WT biotype did not.
- The 96 lines of lettuce and wild species evaluated showed a range of susceptibility to WT and Rb *N. ribisnigri* confirming genetic variation exists for the resistance trait between lines.
- Line 95, *L. virosa* (LJ 10411), was the most resistant to both WT and Rb *N. ribisnigri* and is the original wild donor species of the Nr-gene. Therefore, as Line 95 provided resistance to the Rb biotype, it must contain 'novel' resistance genes, in addition to the Nr-gene. These additional genes must determine a different type of resistance mechanism in the host plant compared to the Nr-gene.
- Line 96 was the second most resistant line to WT *N. ribisnigri*, but other lines were more resistant to the Rb biotype.
- Screening of *C. intybus* and *C. endive* accessions identified Line 50 which showed resistance to Rb *N. ribisnigri* confirming that relatives of lettuce are a source of genetic variation for resistance traits.

- Screening of mapping parents did not identify any lines which provided complete resistance to Rb and WT *N. ribisnigri* but confirmed that Line 96 was still one of the most resistant lines. The parent of the Saladin TILLING population also provided resistance against the WT biotype and screening the subsequent population of this parent could provide offspring with genetic variability for the resistance trait.
- Wild species and relatives are an important source of genetic variability for beneficial traits such as resistance to insects.

Chapter 8: General Discussion

Nasonovia ribisnigri is a serious pest of lettuce, where its presence can lead to unmarketable produce and financial losses for growers. Its significance as a pest is exacerbated by its preference to feed in the centre of lettuce heads, which protects it from the effects of certain insecticides and natural enemies. Furthermore, the effectiveness of current control measures are threatened by the continuous reduction in the number of active ingredients available for insect control, and the development of insecticide resistant and host plant resistance-breaking biotypes, meaning that the need for new methods of control has never been more important.

Prior to this study, little information was available on the biology and behaviour of *N. ribisnigri*, which is essential for the development of control measures and the effective timing of their application. Therefore, the specific objectives of this study set out to provide some of this essential knowledge to aid the development of an integrated pest management strategy and to refine some of the components within it. The objectives set out were as followed:

1) Investigate the effects of photoperiod and temperature on the development of parthenogenetic aphids (Chapter 3).
2) Investigate the conditions required to stimulate development of sexual morphs, egg production, termination of egg diapause and egg hatching (Chapter 4).
3) Investigate alternative host plants (to lettuce) and confirm whether *N. ribisnigri* can use them as overwintering hosts (Chapter 5).
4) Investigate the population dynamics of *N. ribisnigri* in response to natural enemies and entomopathogenic fungi (Chapter 6).
5) Investigate the potential of *Lactuca* species and their relatives to provide new sources of resistance genes which could be used to develop resistant cultivars with new mechanisms of resistance (Chapter 7).

The outcomes of this study and achievement of these objectives will now be addressed in turn:

Investigate the effects of photoperiod and temperature on the development of parthenogenetic summer aphids (Chapter 3).

In Chapter 3, the effects of temperature and photoperiod on the developmental parameters of WT *N. ribisnigri* were described. This included optimum temperatures and lower and upper developmental thresholds (agreeing with those determined in a similar study by Diaz and Fereres (2005). These were determined by using the data collected in this study, which described a linear relationship between development rate and temperature, allowing for the estimation of the day-degree requirements for development from nymph to the final adult moult (which was again similar to those determined by Diaz and Fereres (2007).

Prior to this study, the method used for predicting the population development of *N. ribisnigri* in the UK was based on a day-degree model, using the lower developmental threshold for *P. bursarius* (Collier, *et al.*, 1994). However, this forecast can now be refined, using the values determined specifically for *N. ribisnigri*, to provide a more accurate forecast of its activity.

As this study was constrained by labour and time resources, future work could continue to improve the accuracy of the forecast by increasing the data set used to determine the linear relationship. Investigations could also be made into the use of non-linear models which may describe the relationship better and provide a more accurate forecast. In addition, this study showed that photoperiod did not influence development, and estimates were similar between Rb and WT *N. ribisnigri*, meaning that these factors do not need to be considered in the development of the forecast.

Finally, this study raised questions about the effectiveness of aphid-resistant cultivars at lower temperatures, where the control provided by the Nr-gene appeared to fail. However, as the ambient temperature fluctuates in the field, and is likely to be above 15ºC for at least some of the period during which lettuce crops are grown, resistance will still be provided against WT *N. ribisnigri*. As a breakdown in resistance was not observed in the field prior to the 'arrival' of the new resistance-breaking biotype, it seems likely that the temperature sensitivity of the Nr-gene is unlikely to threaten the control of WT *N. ribisnigri*. Despite this, the effects of temperature, particularly fluctuating temperatures, on the performance of new resistant cultivars should be analysed to clarify this.

Investigate the conditions required to stimulate development of sexual morphs, egg production, termination of egg diapause and egg hatching (Chapter 4).

A phenological forecast of egg development and hatching in the spring would provide important information on the activity of *N. ribisnigri* and indicate when it is likely to migrate to lettuce crops. This information would support growers in deciding when to apply preventative control measures such as crop covers and possibly the use of seed treatments or resistant cultivars, depending on how far in advance *N. ribisnigri* activity can be predicted. The forecast would also be useful if effective control of *N. ribisnigri* could be achieved by controlling developing *N. ribisnigri* fundatrices and offspring on the winter host; whether this would be worthwhile requires further research.

Unfortunately, the data collected during this study were insufficient to support the development of a forecast for egg hatch. However, the study has provided methods to initiate sexual morph production and produce eggs in the laboratory, which were unavailable prior to this study. These methods can now be used in the future to determine the relationship between egg development and temperature, which can be used in turn to estimate the LDT and develop a forecast (Graf, *et al.*, 2006). Monitoring of the winter lifecycle in this study also provided basic information on the overwintering biology of *N. ribisnigri*, confirming the timings of key events such as migration, egg laying and egg hatch.

Investigate alternative host plants (to lettuce) and confirm whether N. ribisnigri can use them as overwintering hosts (Chapter 5).

Various weeds can provide alternative sources of refuge for pests, in addition to their host crop. The data collected in this study confirmed that there are several alternative hosts that WT and Rb *N. ribisnigri* can utilise in the summer. Furthermore, a selection of these host plants also supported overwintering parthenogenetic *N. ribisnigri* between November and March.

The study also confirmed that, should *N. ribisnigri* overwinter as nymphs/adults in the Midlands, as observed in the South of England, they could overwinter successfully, at least in some years. This could have important implications for the

timing of their spring migration to other hosts, with aphids overwintering in the active stages continuing development as soon as temperatures exceed the LDT. It is likely that they would migrate to lettuce crops 'sooner' and develop larger summer populations than those overwintering as eggs. Removal of potential winter host plants would remove possible refuges for *N. ribisnigri* but consideration must be given to their 'other' roles, for example, as a nectar source for natural enemies during the summer.

Finally, this study confirmed that temperature and host plant location were the key factors determining aphid survival during the winter, with a combination of sheltered plants and mild winters resulting in enhanced survival and potentially larger spring populations.

Investigate the population dynamics of N. ribisnigri in response to natural enemies and entomopathogenic fungi (Chapter 6).

The monitoring of *N. ribisnigri* populations during 2010 and 2011 recorded the occurrence of the mid-summer crash, which has been described for various aphid species. In this study, in both years, high natural enemy numbers were observed prior to the decline, suggesting that this was one of the most important regulating factors for *N. ribisnigri* populations. While entomopathogenic fungi, syrphid larvae and parasitoids were present in the highest numbers during these trials, future work should focus on determining the effects of individual predator species through the methods discussed in Chapter 6.

Emigration was also determined to be an important factor regulating aphid population as the numbers of alate aphids were observed to increase prior to the mid-summer crash in both field trial years. As this study only analysed the potential for emigration to occur, future work should implement methods to monitor 'real time' emigration to confirm its role in the mid-summer crash.

As data collection in this project was only performed by one researcher, there were limitations and, unfortunately, the species of parasitoids and fungus specifically affecting *N. ribisnigri* were not recorded. Future work should aim to identify these

natural enemies, as effective biological control agents might be identified which could be introduced or enhanced as part of an IPM strategy.

Like various other studies, this study has failed to identify a single factor which resulted in the mid-summer crash, but it has identified significant factors involved. Due to its complex nature it is uncertain whether the mid-summer crash will ever be understood fully, but achieving this would allow researchers to predict when aphids will decline naturally, therefore avoiding unnecessary insecticide applications. Idealistically, identifying the factors responsible could facilitate the re-creation of these conditions in the field to induce an aphid decline when required.

Investigate the potential of Lactuca species and their relatives to provide new sources of resistance genes which could be used to develop resistant cultivars with new mechanisms of resistance (Chapter 7).

Resistant cultivars are an 'environmentally friendly' method of controlling pest insects and can be used effectively within an integrated pest management programme. Based on a single resistance gene (Nr-gene), conferring complete resistance, the resistance in commercial lettuce cultivars has provided effective control of *N. ribisnigri* until recently. The development of a new resistance-breaking biotype of *N. ribisnigri* has threatened their future.

In October 2009, a sample of *N. ribisnigri,* that had colonised a crop of a resistant variety of lettuce in Kent, was sent to Warwick Crop Centre. This study confirmed that these aphids were the 'new' resistance-breaking biotype, demonstrating, as have previous studies on other pest insects (Buntin, *et al.*, 1990; Eigenbrode, 2002; Kumar, 2005) that single gene 'complete' resistance can be overcome in time and may therefore be only a 'short term' solution. Currently the spread of this new biotype within the UK and its impact on the management of *N. ribisnigri* have not been determined, probably because field infestations of *N. ribisnigri* have been low over the past three years (possibly as a result of unfavourable weather conditions). Interestingly, the insecticide resistant clones of *N. ribisnigri* identified in earlier studies in the UK (Barber, *et al.*, 1999), do not appear to have become more abundant over the last few years, which may again be merely a reflection of

generally low levels of infestation or because the insecticide regimes used in recent times have not provided a high selection pressure for these forms of resistance. .

The potentially short life span of the aphid-resistant cultivars available currently indicates that a 'new' approach is required. One component would be the identification of new sources of resistance that will provide different mechanisms of resistance, this could either be complete resistance or partial resistance that could be used in combination with other methods of control as part of an IPM strategy. This study confirmed that new sources of resistance to both WT and Rb *N. ribisnigri* can be found in wild lettuce and its relatives and these could be exploited to develop new resistant cultivars. This will be particularly important for the development of an IPM strategy to control the new resistance-breaking biotype, should it become widespread. However, as discussed in Chapter 7, future research should focus on encompassing polygenic resistance, which would be more stable and 'challenging' for the aphid to overcome than single gene resistance (Dreyer and Campbell, 1987). Recently, a patent has been filed for the development of new cultivars with resistance to the Rb biotype, originating from *L. serriola* (Teekens, *et al.*, 2010). This resistance has, however, been described as monogenic and therefore is likely to only be a short term solution when used as the main control method, as there would be high selective pressure for the development of resistance-breaking biotypes. If these cultivars were used within an IPM programme in alternation with susceptible lettuce and other control methods, this would reduce the selective pressure and could prolong their life span as an effective control measure.

Life- cycle

Until now, the lifecycle of *N. ribisnigri* has always been described generally with details of its primary and secondary host plants and the timing of its migration between them. By using the information collected in this study a more detailed life-cycle can be provided:

Nasonovia ribisnigri asexually reproduces throughout the summer months on *Lactuca* spp. and other broad leave weeds including *C. intybus, C. capillaris, L. communis, H. aurantiacum, H. pilosella, V. arvensis, V. spicata* and *V. officinalis.*

Development occurs at temperatures above the estimated lower development threshold of 4.7°C, where development from nymph to the final adult moult takes approximately 120 day-degrees with temperatures exceeding 26°C becoming deleterious to development.

As temperature and day length decrease in autumn, alate males are produced initially, followed by alate gynoparaes (observed to be produced together at 12°C 13L:11D), which migrate around mid-October to the winter host (*Ribes* species). The gynoparae then produce female oviparae which lay eggs after mating with males found on the winter host.

Once the eggs have been deposited, usually in the angle between a stem and a bud, they enter a state of diapause, which terminates naturally in the field between late-January and early-February. However, the preponderance of temperatures below the lower developmental threshold for egg development delays hatching until late February.

Once the eggs have hatched, the fundatrices develop and begin reproduction, feeding from the nutrient rich buds of the primary host plant. Once the offspring develop into alate adults, migration to the secondary host occurs and the primary host plant is no longer accepted as a suitable host for colonisation (no nymphs are produced).

It has also been confirmed that in the South of England and the Midlands *N. ribisnigri* can overwinter as active aphids (adults and nymphs) on ‘alternative’ host plants to lettuce, particularly *V. arvensis*.

Appendix

Chapter: General techniques

Table A1 Abbreviation used in this study and their meaning.

Abbreviation	Meaning
BBCH	Biologische Bundesanstalt, Bundessorte namt und Chemische Industrie
CE	Controlled Environment
CL	Confidence Limit
Cv. or Cvs.	Cultivar or Cultivars
DD	Day-degree
Exp.	Experiment
IPM	Integrated Pest Management
IRU	Insect Rearing Unit
LDT	Lower Developmental Threshold
LOG	Logarithm
LSD	Least Significant difference
n	Number of Observations
Rb	Resistance-breaking
RH	Relative Humidity
r_m	Intrinsic Rate of Increase
SE	Standard Error
Sp. or Sp.	Species
T	Total
Tn	Number of Possible Observations
Treat.	Treatment
UDT	Upper Developmental Threshold
WT	Wild-type

Chapter: Host Plant Resistance

Table A2 Line number, name, accession code and crop type of the 96 lines screened from the Vegetable Genetic Improvement Network (VeGIN) at The Warwick Crop Centre, The University of Warwick.

Line Number	Line Name	Accession	Crop type
1	Saladin	LJ 10317	Iceberg
2	Iceberg	LJ 10318	Batavian
3	Michelmore mapping parent	LJ 10319	Wild
4	Stoke	LJ 10320	Cos
5	Batavia Blonde de Paris	LJ 10321	Batavian
6	Lobjoits Green Cos	LJ 10322	Cos
7	Ambassador	LJ 10323	Butterhead
8	Red Granoble	LJ 10324	Batavian
9	Merville de Quatre Saisons	LJ 10325	Butterhead
10	Bloody Warrior	LJ 10326	Cos
11	New Chicken	LJ 10327	Stem
12	Romain de Benicardo	LJ 10328	Cos
13	Lilian	LJ 10329	Butterhead
14	Batavia Tezier	LJ 10330	Batavian
15	Wunder von Stuttgart	LJ 10331	Butterhead
16	Adriatica 2	LJ 10332	Butterhead
17	Webbs Wonderful	LJ 10333	Crisp
18	Waldermann's Dark Green	LJ 10334	Leaf
19	Chinese Stem Lettuce	LJ 10335	Stem
20	*L. saligna*	LJ 10336	Wild
21	*L. saligna*	LJ 10145 (parent)	Wild
22	*L. saligna*	LJ 10338	Wild
23	Moskovskij Parnikovyi	LJ 10339	Butterhead
24	Smaragd	LJ 10340	Butterhead
25	Teli Vajfej	LJ 10341	Butterhead

26	Tardisix	LJ 10342	Butterhead
27	Grosse Brune Tetue; Bruine Trotskop	LJ 10343	Butterhead
28	Hilde	LJ 10344	Butterhead
29	Verdatre	LJ 10345	Butterhead
30	Verte de Cobham; Cobham Green	LJ 10346	Butterhead
31	Wayahead	LJ 10347	Butterhead
32	Kordaat	LJ 10348	Butterhead
33	Joy of the Village	LJ 10349	Butterhead
34	Butterking	LJ 10350	Butterhead
35	Capitan; 541	LJ 10351	Butterhead
36	Cagraner Sommer	LJ 10352	Butterhead
37	Kral Maje I	LJ 10353	Butterhead
38	Bronowicka	LJ 10354	Butterhead
39	Outdoor lettuce	LJ 10355	Butterhead
40	Proftuins Blackpool	LJ 10356	Butterhead
41	Alface (butterhead)	LJ 10357	Butterhead
42	Rudolfs Liebling	LJ 10358	Butterhead
43	*L. sativa* (no name)	LJ 10359	Cos
44	Forellenschluss	LJ 10360	Cos
45	Floricos 83	LJ 10361	Cos
46	*L. sativa* (no name)	LJ 10362	Cos
47	*L. sativa* (no name)	LJ 10363	Cos
48	Kahu	LJ 10364	Cos
49	Romana Larga Catalana	LJ 10365	Cos
50	Yedicule Yagli Marul	LJ 10366	Cos
51	Kaiser Selbstschluss	LJ 10367	Cos
52	Kakichisha White	LJ 10368	Cos
53	*L. sativa* (no name)	LJ 10369	Cos
54	Pallone; Ballon	LJ 10370	Cos
55	Romaine Verte	LJ 10371	Cos
56	Frisee de Beauregard	LJ 10372	Crisp
57	Great Lakes	LJ 10181 (parent)	Crisp

58	L. sativa (no name)	LJ 10374	Crisp
59	Alface (crisp)	LJ 10375	Crisp
60	Aspen; RS822560	LJ 10184 (parent)	Crisp
61	Shladha	LJ 10377	Crisp
62	Prazan	LJ 10378	Crisp
63	Gloire du Dauphine	LJ 10379	Crisp
64	*L. sativa* (no name)	LJ 10380	Crisp
65	White Lettuce	LJ 10381	Cutting
66	Monet; RS867531	LJ 10382	Cutting
67	*L. sativa* (no name)	LJ 10383	Cutting
68	*L. sativa* (no name)	LJ 10384	Cutting
69	*L. sativa* (no name)	LJ 10385	Cutting
70	Amerikanische Brauner	LJ 10386	Cutting
71	Hohlblattringer Butter	LJ 10387	Cutting
72	Krauser Gelber	LJ 10388	Cutting
73	Oak Leaf	LJ 10389	Cutting
74	Simpson	LJ 10390	Cutting
75	Sucrine; Little Gem	LJ 10391	Latin
76	Mestnyi	LJ 10392	Latin
77	Bibb	LJ 10393	Latin
78	Alface Repolho	LJ 10394	Latin
79	Okayama Salad	LJ 10395	Latin
80	Deer Tongue	LJ 10396	Latin
81	Midget Cos	LJ 10397	Latin
82	Balady	LJ 10398	Oilseed
83	*L. sativa* (no name)	LJ 10399	Stalk
84	*L. serriola*	LJ 10400	Wild
85	*L. serriola*	LJ 10401	Wild
86	*L. serriola*	LJ 10210 (parent)	Wild
87	*L. serriola*	LJ 10211 (parent)	Wild
88	*L. serriola*	LJ 10404	Wild
89	*L. serriola*	LJ 10405	Wild
90	*L. serriola*	LJ 10214 (parent)	Wild

91	*L. serriola*	LJ 10215 (parent)	Wild
92	*L. serriola*	LJ 10408	Wild
93	*L. serriola*	LJ 10409	Wild
94	*L. serriola*	LJ 10410	Wild
95	*L. virosa*	LJ 10411	Wild
96	*L. virosa*	LJ 10220 (parent)	Wild

Table A3 Line number, name, species and accession code of *Cichorium intybus* (14) and *Cichorium endivia* (18) lines screened.

Line Num.	**Line Name**	**Species**	**Accession code**
3	Di verona (Red)	*C. intybus*	3225
6	Cicoria O Radicchio selvatica da campo	*C. intybus*	5216
7	Cicoria O Radicchio da taglio a foglia	*C. intybus*	5217
9	Cicoria pan di zucchero	*C. intybus*	5219
10	Indivia romanesca da taglio	*C. endivia*	5221
13	Indivia ricciuta di pancalieri	*C. endivia*	5224
16	Di verona rossa a palla	*C. intybus*	5242
19	Breedblad volhart winter brevo Rs	*C. endivia*	5605
20	Malan	*C. endivia*	5606
22	De ruffec rocco	*C. endivia*	5608
25	Kwarosa	*C. intybus*	5968
26	Varigata di sottomarina	*C. intybus*	6529
27	Cicoria rossa di treviso	*C. intybus*	6530
30	No name	*C. intybus*	7223
31	Chicori	*C. intybus*	12117
32	Amelioree pain de sucre race elmo	*C. intybus*	12721
33	Marly	*C. endivia*	12722
35	Diva	*C. intybus*	12941
36	Vilmorin No 5	*C. intybus*	12979
37	Tosca	*C. endivia*	12981

38	Traviata	*C. endivia*	12980
39	Atria	*C. endivia*	12998
40	Cornelia	*C. endivia*	12999
41	Sacha	*C. endivia*	13000
43	Ariga	*C. endivia*	13002
44	Dorana	*C. endivia*	13003
45	Sally	*C. endivia*	13004
46	Glory	*C. endivia*	13006
47	Gilda	*C. endivia*	13007
48	Minerva	*C. endivia*	13008
49	Lea	*C. endivia*	13010
50	Bea (F1)	*C. intybus*	13019

References

Aarts, R., Schut, J.W., Driessen, R. and Reinink, K. (1999). Integrated control for aphids on lettuce varieties resistant to *Nasonovia ribisnigri. Mededelingen Faculteit Landbouwkundige Toegepaste Biologische Wetenschappen,* **64/3**, 11-15.

Abdullah, M. (1961). Behavioural effects of temperature on insects. *The Ohio Journal of Science,* **61**, 212-219.

Acreman, S.J. and Dixon, A.F.G. (1989). The effects of temperature and host quality on the rate of increase of the grain aphid (*Sitobion avenae*) on wheat. *Annals of Applied Biology,* **115**, 3-9.

Aldyhim, Y.N. and Khalil, A.F. (1993). Influence of temperature and daylength on population development of *Aphis gossypii* on *Cucurbita pepo. Entomologia Experimentalis et Applicata,* **67**, 167-172.

Alkhedir, H., Karlovsky, P. and Vidal, S. (2010). Effect of light intensity on colour morph formation and performance of the grain aphid *Sitobion avenae* F. (Homoptera: Aphididae). *Journal of Insect Physiology*, **56**, 1999-2005.

Alyokhin, A., Drummond, F.A., Sewell, G. and Storch, R.H. (2011). Differential effects of weather and natural enemies on coexisting aphid populations. *Environmental Entomology*, **40**, 570-580.

Ameixa, O.M.C.C. and Kindlmann, P. (2011). Some exclusion cages do not exclude predators. *European Journal of Environmental Sciences,* **1**, 67-69

Anderson, K.G. and Harris, M.O. (2006). Does R gene resistance allow wheat to prevent plant growth effects associated with Hessian fly (Diptera: Cecidomyiidae) attack? *Journal of Economic Entomology,* **99**, 1842-1853.

APG. (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society,* **161**, 105-121.

Arnold, C.Y. (1959). The determination and significance of the base temperature in a linear heat unit system. *Proceedings of the American Society of Horticultural Science*, **4**, 430-445.

Awmack, C.S. and Leather, S.R. (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology,* **47**, 817-844.

Awmack, C.S. and Leather, S.R. (2007). Growth and Development. In: *Aphids as Crop Pests,* pp. 135-151. Edited by H.F. van Emden and R. Harrington. Wallingford: CABI Publishing.

Babura, S.R. and Mustapha, Y. (2012). Screening for development of host plant resistance to infestation by aphid (*Aphis craccivora* Koch) in cowpea (*Vigna unguiculata* [L] Walp). *Bayero Journal of Pure and Applied Sciences*, **5**, 44-47.

Bale, J.S. and Hayward, S.A.L. (2010). Insect overwintering in a changing climate. *The Journal of Experimental Biology*, **213**, 980-994.

Barber, M.D., Moores, G.D., Denholm, I., Kift, N.B. and Tatchell, G.M. (2002). Resistance to insecticides in the currant-lettuce aphid, Nasonovia ribisnigri: laboratory and field evidence. *The BCPC Conference: Pests and diseases, Volumes 1 and 2. Proceedings of an international conference held at the Brighton Hilton Metropole Hotel.* Brighton, UK, 18-21 November 2002, pp. 817-822.

Barber, M.D., Moores, G.D., Tatchell, G.M., Vice, W.E. and Denholm, I. (1999). Insecticide resistance in the currant-lettuce aphid, *Nasonovia ribisnigri* (Hemiptera : Aphididae) in the UK. *Bulletin of Entomological Research*, **89**, 17-23.

Barlow, C.A. (1962). Development, survival, and fecundity of the potato aphid, *Macrosiphum euphorbiae* (Thomas), at constant temperatures. *The Canadian Entomologist*, **94**, 667-671.

Baskerville, G.L. and Emin, P. (1969). Rapid estimation of heat accumulation from maximum and minimum temperatures. *Ecology*, **50**, 514-517.

Basky, Z. (1993). Incidence and population fluctuations of *Diuraphis noxia* in Hungary. *Crop Protection* **12**, 605-609.

Basky, Z. (2003). Predators and parasitoids on different cereal aphid species under caged and no caged conditions in Hungary. *Proceedings of the 8th Internation Symposium on Ecology of Aphidophaga.* Ponta Delgada, Portugal, pp. 95-96.

Bayer. (2011). *Environmental Information Sheet Decis® MAPP 07172*. [online] Bayer CropScience. Available at: http://www.bayercropscience.co.uk/assets/Document-Store/EIS/Decis-Version-3-Guideline-version-4.pdf [Accessed: 20 January 2010].

Becerra, J.X. (1997). Insects in plants: macroevolutionary chemical trends in host use. *Science*, **276**, 253-256.

Becerra, J.X. and Venable, D.L. (1999). Macroevolution of insect-plant associations: the relevance of host biogeography to host affiliation. *Proceedings of the National Academy of Sciences*, **96**, 12626-12631.

Bianchi, F.J.J.A. and Wäckers, F.L. (2008). Effects of flower attractiveness and nectar availability in field margins on biological control by parasitoids. *Biological Control*, **46**, 400-408.

Blackman, R.L. (1971). Variation in the photoperiodic response within natural populations of *Myzus persicae* (Sulz.). *Bulletin of Entomological Research*, **60**, 533-546.

Blackman, R.L. (1974). Life-cycle variation of *Myzus persicae* (Sulz.) (Hom., Aphididae) in different parts of the world, in relation to genotype and environment. *Bulletin of Entomological Research*, **63**, 595-607.

Blackman, R.L. (2010). *Aphids- Aphidinae (Macrosiphini).* Volume 2 part 7. Shrewsbury: Royal Entmological Society.

Blackman, R.L. and Eastop, V.F. (1984). *Aphids on the World's Crops, An Identification Guide*. First edn. Chichester: John Wiley & Sons.

Blackman, R.L. and Eastop, V.F. (2000). *Aphids on the World's Crops: An Identification and Information Guide*. Second edn. Chichester: John Wiley & Sons.

Blackman, R.L. and Eastop, V.F. (2006a). *Aphids on the World's Herbaceous Plants and Shrubs.* Volume 1 Host Lists & Keys. Chichester: John Wiley & Sons.

Blackman, R.L. and Eastop, V.F. (2006b). *Aphids on the World's Herbaceous Plants and Shrubs.* Volume 2 The Aphids. Chichester: John Wiley & Sons Ltd.

Blackman, R.L. and Eastop, V.F. (2007). Taxonomic Issues. In: *Aphids as Crop Pests*, pp. 1-29. Edited by H.F. van Emden and R. Harrington. Wallingford: CABI Publishing.

Bommarco, R. and Ekbom, B. (1996). Variation in pea aphid population development in three different habitats. *Ecological Entomology*, **21**, 235-240.

Braendle, C., Davis, G.K., Brisson, J.A. and Stern, D.L. (2006). Wing dimorphism in aphids. *Heredity*, **97**, 192-199.

Braendle, C., Friebe, I., Caillaud, M.C. and Stern, D.L. (2005). Genetic variation for an aphid wing polyphenism is genetically linked to a naturally occuring wing polyphenism. *Proceedings of The Royal Society B: Biological Sciences*, **272**, 657-664.

Broadbent, L. (1948a). Methods of recording aphid populations for use in research on potato virus diseases. *Annals of Applied Biology*, **35**, 551-566

Broadbent, L. (1948b). Aphis migration and the efficiency of the trapping method. *Annals of Applied Biology*, **35**, 379-394.

Brosius, T.R., Higley, L.G. and Hunt, T.E. (2007). Population dynamics of soybean aphid and biotic mortality at the edge of its range. *Journal of Economic Entomology*, **100**, 1268-1275.

Brück, E., Elbert, A., Fischer, R., Krueger, S., Kühnhold, J., Klueken, A.M., Nauen, R., Niebes, J., Reckmann, U., Schnorbach, H., Steffens, R. and van Waetermeulen, X. (2009). Movento®, an innovative ambimobile insecticide for sucking insect pest control in agriculture: Biological profile and field performance. *Crop Protection*, **28**, 838-844.

Bugg, R.L., Colfer, R.G., Chaney, W.E., Smith, H.A. and Cannon, J. (2008). *Flower flies (Syrphidae) and other biological control agents for aphids in vegetable crops*. University of California Agriculture and Natural Resources Publication 8285, 1-25.

Buntin, G.D., Bruckner, P.L., Johnson, J.W. and Foster, J.E. (1990). Effectiveness of selected genes for hessian fly resistance in wheat. *Journal of Argicultural Entomology*, **7**, 283-281.

Butts, R.A. and F.L., McEwen (1981). Seasonal populations of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) in relation to day-degree accumulation. *The Canadian Entomologist*, **113**, 127-131.

CABI/EPPO. (2003). *Nasonovia ribisnigri*. *[Distribution map]*. CABI Distribution Maps of Plant Pests, 1st Edition, Map 644.

Caldwell, B., Brown Rosen, E., Sideman, E., Shelton, A. and Smart, C. (2005). Appendix A: Plant resistance to insects and diseases. In: *Resource guide for organic insect and disease management, pp.*147-149. Edited by B. Caldwell, E. Brown Rosen, E. Sideman, A. Shelton and C. Smart. New York: New York State Agricultural Experiment Station.

Campbell, A., Frazer, B.D., Gilbert, N., Gutierrez, A.P. and Mackauer, M. (1974). Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology*, **11**, 431-438.

Campbell, A. and Mackauer, M. (1977). Reproduction and population growth of the pea aphid (Homoptera: Aphididae) under laboratory and field conditions. *The Canadian Entomologist*, **109**, 277-284.

Campbell, B.C., Jones, K.C. and Dreyer, D.L. (1986). Discriminative behavioral responses by aphids to various plant matrix polysaccharides. *Entomologia Experimentalis et Applicata*, **41**, 17-24.

Cannon, R.J.C. (1984). The development rate of *Metapolophium dirhodum* (Walker) (Hemiptera: Aphididae) on winter wheat. *Bulletin of Entomological Research*, **74**, 33-46.

Cardinale, B.J., Harvey, C.T., Gross, K. and Ives, A.R. (2003). Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest supression and crop yield in an agroecosystem. *Ecology Letters*, **6**, 857-865.

Carvalho, G.R., Maclean, N., Wratten, S.D., Carter, R.E. and Thurston, J.P. (1991). Differentiation of aphid clones using DNA fingerprints from

individual aphids. *Proceedings of The Royal Society B: Biological Sciences,* **243**, 109-114.

Casey, T.M. (1992). Biophysical ecology and heat exchange in insects. *American Zoology,* **32**, 225-237.

Chacón, J.M. and Heimpel, G.E. (2010). Density-dependent intraguild predation of an aphid parasitoids. *Oecologia,* **164**, 213-220.

Chambers, R.J., Sunderland, K.D., Wyatt, I.J. and Vickerman, G.P. (1983). The effects of predator exclusion and caging on cereal aphids in winter wheat. *Journal of Applied Ecology,* **20**, 209-224.

Chandler, A.E.F. (1968). The relationship between aphid infestations and oviposition by aphidophagous Syrphidae (Diptera). *Annals of Applied Biology,* **61**, 425-434.

Chown, S.L. and Nicolson, S.W. (2004). *Insect Physiological Ecology: mechanisms and patterns*. Oxford: Oxford University Press.

Chuche, J. and Thiéry, D. (2009). Cold winter temperatures condition the egg-hatching dynamics of a grape disease vector. *Naturwissenschaften,* **96**, 827-834.

Cognetti, G. (1961). Endomeiosis in parthenogenetic lines of aphids. *Experientia,* **17,** 168-169.

Collier, R.H. (2007a). *The biology and control of currant-lettuce aphid (Nasonovia ribisnigri).* Horticultural Development Company. Final report project number FV279.

Collier, R.H. (2007b). *The impact of climate change on pests of horticultural pests.* Commercial Grower.

Collier, R.H. and Finch, S. (1983). Effects of intensity and duration of low temperatures in regulating diapause development of the cabbage root fly (*Delia radicum*). *Entomologia Experimentalis et Applicata,* **34**, 193-200.

Collier, R.H. and Finch, S. (1985). Accumulated temperatures for predicting the time of emergence in the spring of the cabbage root fly, *Delia radicum* (L.) (Diptera: Anthomyiidae). *Bulletin of Entomological Research,* **75**, 395-404.

Collier, R.H. and Finch, S. (2001). Forecasting attacks by pest insects of cruciferous crops. *Proceedings of the 4th International Workshop: The management of diamondback moth and other crucifer pests.* Melbourne, Australia, 26-29 November 2001, pp. 163-168.

Collier, R.H. and Harrington, R. (2001). Outdoor lettuce: Refinement and field validation of forecasts for the aphid pests of lettuce foilage. Final Report Project Number FV162. Horticultural Development Company.

Collier, R.H., Tatchell, G.M., Ellis, P.R. and Parker, W.E. (1999). Strategies for the control of aphid pests of lettuce. *IOBC/WPRS Bulletin,* **22**, 25-35.

Collier, R.H., Davies, J.A., Roberts, M., Leatherland, M., Runham, S.R. and Blood Smyth, J.A. (1994). Monitoring and forecasting the times of attack of the lettuce root aphid, *Pemphigus bursarius* L. *IOBC/WPRS Bulletin*, **17**, 31-40.

Costamagna, A.C. and Landis, D.A. (2007). Quantifying predation on soybean aphid through direct field observations. *Biological Control,* **42**, 16-24.

Cox, C. (2004). Managing aphid problems without pesticides. *Journal of Pesticide Reform,* **24**, 8-9.

Crawley, M.J. (1983). *Herbivory: the dynamics of animal-plant interactions*. Oxford: John Wiley and Sons.

Cuthbertson, A.G.S., Mathers, J.J. and Northing, P. (2007). A Preliminary Investigation into chemical efficacy against the currant-lettuce aphid *Nasonovia ribisnigri. International Journal of Agricultural Research,* **8**, 741-744.

Damos, P. and Savopoulou-Soultani, M. (2012). Temperature-driven models for insect development and vital thermal requirements. *Psyche,* **2012**, 1-13.

Davis, J.A., Radcliffe, E.B. and Ragsdale, D.W. (2006). Effects of high and fluctuating temperatures on *Myzus persicae* (Hemiptera: Aphididae). *Environmental Entomology,* **35**, 1461-1468.

Davis, R.M., Subbarao, K.V., Raid, R.N. and Kurtz, E.A. (1997). *Compendium of lettuce diseases*. Minnesota: The American Phytopathological Society.

de Conti, B.F., Bueno, V.H.P., Sampaio, M.V. and Sidney, L.A. (2010). Reproduction and fertility life table of three aphid species (Macrosiphini) at different temperatures. *Revista Brasileira de Entomologia,* **54**, 654-660.

de Vries, I.M. (1997). Origin and domestication of *Lactuca sativa* L. *Genetic Resources and Crop Evolution,* **44**, 165-174.

De-silva, D.L., Vásquez, A.S. and Mallet, J. (2011). Selection for enemy-free space: eggs placed away from the host plant increase survival of a neotropical ithomiine butterfly. *Ecological Entomology,* **36**, 667-672.

Dean, G.J. (1974). Effect of temperature on the cereal aphids *Metopolophium dirhodum* (Wlk.), *Rhopalosiphum padi* (L.) and *Macrosiphum avenue* (F.) (Hem., Aphididae). *Bulletin of Entomological Research,* **63**, 401-409.

Dedryver, C., Le Ralec, A. and Fabre, F. (2010). The conflicting relationships between aphids and men: A review of aphid damage and control strategies. *Comptes Rendus Biologies,* **333**, 539-553.

DEFRA. (2005). The modelling of the key parameters to measure the potential of a parasitoid to control the aphid Nasonovia ribisnigri. Final Report Project Number HH3119TPC.

Denlinger, D.L. (2002). Regulation of diapause. *Annual Review of Entomology*. **47**, 93-122.

Dent, D. (2000). *Insect Pest Management*. Wallingford: CABI Publishing.

Dewar, A.M. (2007). Chemical Control. In: *Aphids as Crop Pests*, pp 391-422. Edited by H.F van Emden and R. Harrington. Wallingford: CABI Publishing

Dewar, A.M., Dean, G.J. and Cannon, R. (1982). Assessment of methods for estimating the numbers of aphids (Hemiptera: Aphididae) in cereals. *Bulletin of Entomological Research* **72**, 675-685.

Diaz, B.M. and Fereres, A. (2005). Life table and population parameters of *Nasonovia ribisnigri* (Homoptera : Aphididae) at different constant temperatures. *Environmental Entomology,* **34**, 527-534.

Diaz, B.M., Barrios, L. and Fereres, A. (2012). Interplant movement and spatial distribution of alate and apterous morphs of *Nasonovia ribisnigri* (Homoptera: Aphididae) on lettuce. *Bulletin of Entomological Research,* **31**, 1-9.

Diaz, B.M., Muñiz, M., Barrios, L. and Fereres, A. (2007). Temperature thresholds and thermal requirements for development of *Nasonovia ribisnigri* (Hemiptera : Aphididae). *Environmental Entomology,* **36**, 681-688.

Diehl, E., Sereda, E., Wolters, V. and Birkhofer, K. (2013). Effect of predator specialization, host plant and climate on biological control of aphids by natural enemies: a meta-analysis. *Journal of Applied Ecology,* **50**, 262-270.

Dixon, A.F.G. (1976). Timing of egg hatch and viability of the sycamore aphid, *Drepanosiphum platanoidis* (Schr.), at bud burst of sycamore, *Acer pseudoplatanus* L. *Journal of Animal Ecology*, **45**, 593-603.

Dixon, A.F.G. (1977). Aphid ecology: life-cycles, polymorphism, and population Regulation. *Annual Review of Ecology and Systematics*, **8**, 329-353.

Dixon, A.F.G. (1987a). Seasonal Development in Aphids. In: *Aphids; their biology, natural enemies and control*, *World Crop Pests, Vol. 2a,* pp. 315-320. Edited by A.K. Minks, and P. Harrewijn. Amsterdam: Elsevier.

Dixon, A.F.G. (1987b). Parthenogenetic Reproduction and the Rate of Increase in Aphids. In: *Aphids; their biology, natural enemies and control*, *World Crop Pests, Vol. 2a,* pp. 269-287. Edited by A.K. Minks, and P. Harrewijn. Amsterdam: Elsevier.

Dixon, A.F.G. (1987c). The way of life of aphids: Host specificity, speciation and distribution. In: *Aphids; their biology, natural enemies and control, World Crop Pests, Vol. 2a,* pp. 197-207. Edited by A.K. Minks, and P. Harrewijn. Amsterdam: Elsevier.

Dixon, A.F.G. (1998). *Aphid Ecology: An Optimization Approach*. 2nd edn. London: Chapman and Hall.

Dixon, A.F. and Dewar, A.M. (1974). The time of determination of gynoparae and males in the bird cherry-oat aphid, *Rhopalosiphum padi. Annals of Applied Biology,* **78**, 1-6.

Dixon, A.F.G. and Hopkins, G.W. (2010). Temperature, Seasonal Development and Distribution of Insects with Particular Reference to Aphids. In: *Aphid biodiversity under environmental change: patterns and processes*, pp. 129-147. Edited by P. Kindlmann, A.F.G. Dixon, and J.P. Michaud. Dordrecht: Springer.

Dixon, A.F.G. and Kundu, R. (1994). Ecology of host alternation in aphids. *European Journal of Entomology*, **91**, 63-70.

Dixon, A.F.G. and Wratten, S.D. (1971). Laboratory studies on aggregation, size and fecundity in the black bean aphid, *Aphis fabae* Scop. *Bulletin of Entomological Research,* **61**, 97-111.

Dixon, A.F.G., Horth, S. and Kindlmann, P. (1993a). Migration in insects: cost and strategies. *Journal of Animal Ecology*, **62**, 182-190.

Dixon, A.F.G., Wellings, P.W., Carter, C. and Nichols, J.F.A. (1993b). The role of food quality and competition in shaping the seasonal cycle in the reproductive activity of the sycamore aphid. *Oceologia*, **95**, 89-92.

Dogimont, C., Bendahmane, A., Chovelon, V. and Boissot, N. (2010). Host plant resistance to aphids in cultivated crops: genetic and molecular bases, and interactions with aphid populations. *Comptes Rendus Biologies,* **333**, 566-573.

Donaldson, J.R., Myers, S.W. and Gratton, C. (2007). Density-dependent responses of soybean aphid (*Aphis glycines* Matsumura) populations to generalist predators in mid to late season soybean fields. *Biological Control*, **43**, 111-118.

Douglas, A.E. (2003). The nutritional physiology of aphids. *Advances in Insect Physiology*, **31**, 73-140.

Dreyer, D.L. and Campbell, B.C. (1987). Chemical basis of host-plant resistance to aphids. *Plant, Cell & Environment,* **10**, 353-361.

Dry, W.W and Taylor, L.R. (1970). Light and temperature thresholds for take-off by aphids. *Journal of Animal Ecology,* **39**, 493-504.

Duetting, P.S., Ding, H., Neufeld, J. and Eigenbrode, S.D. (2003). Plant waxy bloom on peas affects infection of pea aphids by *Pandora neoaphidis. Journal of Invertebrate Pathology,* **84**, 149-158.

Dunn, J.A. and Wright, D.W. (1955). Overwintering egg populations of the pea aphid in East Anglia. *Bulletin of Entomological Research,* **46**, 389-392.

Eckenrode, C.J. and Chapman, R.K. (1972). Seasonal adult cabbage maggot populations in the field in relation to thermal-unit accumulations. *Annals of the Entomological Society of America,* **61**, 151-156.

Edwards, C.A., Sunderland, K.D. and George, K.S. (1979). Studies on polyphagous predators of cereal aphids. *Journal of Applied Ecology,* **16**, 811-823.

Edwards, O.R. (2001). Interspecific and intraspecific variation in the performance of three pest aphid species on five grain legume hosts. *Entomologia Experimentalis et Applicata,* **100**, 21-30.

Eenink, A.H. and Dieleman, F.L. (1980). Development of *Myzus persicae* on a partially resistant and on a susceptible genotype of lettuce (*Lactuca sativa*) in relation to plant age. *Netherlands Journal of Plant Pathology,* **86**, 111-116.

Eenink, A.H. and Dieleman, F.L. (1983). Inheritance of Resistance to the leaf aphid *Nasonovia Ribis-nigri* in the wild lettuce species *Lactuca Virosa. Euphytica,* **32**, 691-695.

Eenink, A.H., Dieleman, F.L. and Groenwold, R. (1982a). Resistance of lettuce (*Lactuca*) to the leaf aphid *Nasonovia Ribis Nigri*. 2. Inheritance of the resistance. *Euphytica,* **31**, 301-304.

Eenink, A.H., Groenwold, R. and Dieleman, F.L. (1982b). Resistance of lettuce (Lactuca) to the leaf aphid *Nasonovia Ribis Nigri* .1. Transfer of resistance from *L. Virosa* to *L. Sativa* by interspecific crosses and selection of resistant breeding lines. *Euphytica,* **31**, 291-300.

Eigenbrode, S.D. (2002). Resistance to host-plant resistance. In: *Encyclopedia of pest management* Vol **1** pp. 712-714. Edited by D. Pimentel. NewYork: Taylor and Francis.

Eigenbrode, S.D. and Trumble, J.T. (1994). Host plant resistance to insects in Integrated Pest Management in vegetable crops. *Journal of Agricultural Entomology,* **11**, 201-224.

el Bouhssini, M. and Nachit, M.M. (2000). New sources of resistance in durum wheat and wild relatives to Russian wheat aphid (Homoptera: Aphididae). *Options Méditerranéenes,* **40**, 393-395.

Elsoms. (2011). Vegetable and Seed Catalogue 2011/2012. Spalding: Elsoms Seeds.

Feller, C., Bleiholder, H., Buhr, L., Hack, H., Hess, M., Klose, R., Meier, U., Stauss, R., van den Boom, T. and Weber, E. (1995). Phänologische Entwicklungsstadien von Gemüsepflanzen: I. Zwiebel-, Wurzel-, Knollen- und Blattgemüse. *Nachrichtenbl. Deut. Pflanzenschutzd,* **47**, 193–206.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution,* **39**, 783-791.

Ferguson, K.L. and Stiling, P. (1996). Non-additive effects of multiple natural enemies on aphids populations. *Oecologia,* **108**, 375-379.

Ferrari, J., Müller, C.B., Kraaijeveld, A.R. and Godfray, H.C. (2001). Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution,* **55**, 1805-1814.

Finch, S., Collier, R.H. and Phelps, K. (1996). A review of work done to forecast pest insect attacks in UK horticultural crops. *Crop Protection,* **15**, 353-357.

Finlay, K.J. and Luck, J.E. (2011). Response of the bird cherry-oat aphid (*Rhopalosiphum padi*) to climate change in relation to its pest status, vectoring potential and function in a crop-vector-virus pathosystem. *Agriculture, Ecosystems and Environment,* **144**, 405–421.

Foster, S.P., Denholm, I., Poppy, G.M., Thompson, R. and Powell, W. (2011). Fitness trade-off in peach-potato aphids (*Myzus persicae*) between insecticide resistance and vulnerability to parasitoid attack at several spatial scales. *Bulletin of Entomological Research,* **101**, 659-666.

Foster, S.P., Harrington, R., Devonshire, A.L., Denholm, I., Clark, S.J. and Mugglestone, M.A. (1997). Evidence for a possible fitness trade-off between insecticide resistance and the low temperature movement that is essential for survival of UK populations of *Myzus persicae* (Hemiptera: Aphididae). *Bulletin of Entomological Research,* **87**, 573-579.

Foster, S.P., Harrington, R., Devonshire, A.L., Denholm, I., Devine, G.J., Kenward, M.G. and Bale, J.S. (1996). Comparative survival of insecticide-susceptible and resistance peach-potato aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), in low temperature field trials. *Bulletin of Entomological Research,* **86**, 17-27.

Foster, S.P., Tomiczek, M., Thompson, R., Denholm, I., Poppy, G., Kraaijeveld, A.R. and Powell, W. (2007). Behavioural side-effects of insecticide resistance in aphids increase their vulnerability to parasitoid attack. *Animal Behaviour,* **74**, 621-632.

Frazer, B.D., Gilbert, N., Ives, P.M. and Raworth, D.A. (1981a). Predator reproduction and the overall predator-prey relationship. *The Canadian Entomologist,* **113**, 1015-1024.

Frazer, B.D., Gilbert, N., Nealis, V. and Raworth, D.A. (1981b). Control of aphid density by a complex of predators. *The Canadian Entomologist,* **113**, 1035-1041.

Gange, A.C. and Llewellyn, M. (1988). Egg distribution and mortality in the alder aphid, *Pterocallis alni. Entomologia Experimentalis et Applicata,* **48**, 9-14.

Gao, L., Klinger, J.P., Anderson, J.P., Edwards, O.R. and Singh, K.B. (2008). Characterization of pea aphid resistance in *Medicago truncatula. Plant Physiology,* **146**, 996-1009.

GardenAction. (2010). *Propagating blackcurrant bushes*. [Online]. Available at: http://www.gardenaction.co.uk/fruit_veg_diary/blackcurrant_page3.asp [Accessed: 21 July 2010]

Garthwaite, D.G., Barker, I., Parrish, G., Smith, L., Hudson, S. and Pietravalle, S. (2011). *Pesticide usage survey report- outdoor vegetable crops in the United Kingdom*. The Food and Environment Research Agency.

Garthwaite, D.G., Thomas, M.R., Heywood, E., Battersby, A., Parrish, G. and Smith, L. (2007). *Pesticide usage survey report- outdoor vegetable crops in Great Britain*. The Food and Environment Research Agency.

Gillespie, M., Wratten, S., Sedcole, R. and Colfer, R. (2011). Manipulating floral resources dispersion for hoverflies (Diptera: Syrphidae) in a California lettuce agro-ecosystem. *Biological Control*, **59**, 215–220.

Girousse, C., Moulia, B., Silk, W. and Bonnemain, J.-L. (2005). Aphid infestation causes different changes in carbon and nitrogen allocation in alfalfa stems as well as different inhibition of longitudinal and radial expansion. *Plant Physiology,* **137**, 1474-1484.

Goggin, F.L. (2007). Plant-aphid interactions: molecular and ecological perspective. *Current Opinion in Plant Biology*, **10**, 399-408.

Graf, B., Höpli, H.U., Höhn, H. and Samietz, J. (2006). Temperature effects on egg development of the rosy apple aphid and forecasting of egg hatch. *Entomologia Experimentalis et Applicata,* **119**, 207-211.

Griffiths, D.C. (1960). The behaviour and specificity of *Monoctonus paludum* Marshall* (Hym., Braconidae), a parasite of *Nasonovia ribis-nigri* (Mosley) on lettuce. *Bulletin of Entomological Research,* **51**, 303-319.

Griffiths, E. and Wratten, S.D. (1979). Intra- and inter-specific differences in cereal aphid low-temperature tolerance. *Entomologia Experimentalis et Applicata,* **26**, 161-167.

Groeters, F.R. (1989). Geographic and clonal variation in the milkweed-oleander aphid, *Aphis nerii* (Homoptera: Aphididae) for winged morph production, life

history, and morphology in relation to the host plant permanence. *Evolutionary Ecology,* **3**, 327-341.

Guldemond, J.A., van den Brink, W.J. and den Belder, E. (1998). Methods of assessing population increase in aphids and the effect of growth stage of the host plant on population growth rates. *Entomologia Experimentalis et Applicata,* **86**, 163-173.

Gutiérrez-Ibáñez, C., Villagra, C.A. and Niemeyer, H.M. (2007). Pre-pupation behaviour of the aphid parasitoid *Aphidius ervi* (Haliday) and its consequences for pre-imaginal learning. *Naturwissenschaften,* **94**, 595-600.

Gwynn, D.M., Callaghan, A., Gorham, J., Walters, K.F.A. and Fellowes, M.D.E. (2005). Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid. *Proceedings of the Royal Society B: Biological Sciences,* **272**, 1803-1808.

Haahr, M. (1998). *Random.org- sequence generator*. [Online]. Available at: http://www.random.org/lists/ [Accessed: June-September 2010)

Hahn, D.A. and Denlinger, D.L. (2011). Energetics of insect diapause. *Annual Review of Entomology,* **56**, 103-121.

Hand, S.C. (1983). The effect of temperature and humidity on the duration of development and hatching success of eggs of the aphid, *Sitobion avenae. Entomologia Experimentalis et Applicata,* **33**, 220-222.

Hardie, J. (1990). The photoperiodic counter, quantitative day-length effects and scotophase timing in the vetch aphid *Megoura viciae. Journal of Insect Physiology,* **36,** 939-949.

Hardie, J. (2009). Photoperiodism in insects: Aphids polyphenism. In: *Photoperiodism: The biological calender,* pp. 600. Edited by R.J. Nelson, D.L. Delinger and D.E. Somers. New York: Oxford University Press.

Harrington, R. (1984). Photoperiodic control of sexual morph production by the currant-sowthistle aphid, *Hypetomyzus lactucae. Entomologia experimentalis et Applicata,* **35**, 169-175.

Harrington, R. and Woiwod, I. (2007). Foresight from hindsight: The Rothamsted Insect Survey *Outlooks on Pest Management,* **18**, 9-14.

Harrington, R. and Xia-Nian. C. (1984). Winter mortality, development and reprodution in a field population of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) in England. *Bulletin of Entomological Research,* **74**, 633-640.

Harrington, R., Hulle, M. and Plantegenest. (2007). Monitoring and Forecasting. In: *Aphids as Crop Pests,* pp. 515-536. Edited by H.F. van Emden and R. Harrington. Wallingford: CABI Publishing

Hatano, E., Kunert, G. and Wolfgang, W.W. (2010). Aphid wing induction and ecological costs of alarm pheromone emission under field conditions. *PLoS One,* **5**, 1-5.

Hazell, S.P., Gwynn, D.M., Ceccarelli, S. and Fellowes, M.D.E. (2005). Competition and dispersal in the pea aphid: clonal variation and correlations across traits. *Ecological Entomology,* **30**, 293-298.

HDC. (2009). *Weed Identification Guide*. Kenilworth: Horticultural Development Company.

HDC. (No date). *HDC Pest Bulletin*. [Online]. Syngenta. <http://www3.syngenta.com/country/uk/en/AgronomyTools/HDCPestBulletin/Pages/HDCPestBulletin.aspx> [Accessed: 21 January 2013].

Herms, D.A. (2004). Using Degree-Days and Plant Phenology to Predict Pest Activity. pp. 49-59. In: *IPM (Integrated Pest Management) of Midwest Landscapes*. Edited by V. Krischik and J. Davidson. Minnesota: Agricultural Experiment Station.

Higley, L.G., Pedigo, L.P. and Ostlie, K.R. (1986). DEGDAY: A program for calculating degree-days, and assumptions behind the degree-day approach. *Environmental Entomology,* **15**, 999-1016.

Hirose, Y. (2006). Biological control of aphids and coccids: a comparative analysis. *Population Ecology,* **48**, 307-315.

Hopper, J.V., Nelson, E.H., Daane, K.M. and Mills, N.J. (2011). Growth, development and consumption by four syrphid species associated with the lettuce aphid, *Nasonovia ribisnigri,* in California. *Biological Control,* **58**, 271-276.

Hopper, K.R. (1999). Risk-spreading and bet-hedging in insect population biology. *Annual Review of Entomology,* **44**, 535-560.

Hughes, R.D. (1963). Populations dynamics of the cabbage aphid, *Brevicoryne brassicae* (L.). *Journal of Animal Ecology,* **32**, 393-424.

Hutchison, W.D. and Hogg, D.B. (1984). Demographic statistic for the pea aphid (Homoptera: Aphididae) in Wisconsin and a comparison with other populations. *Environmental Entomology,* **13**, 1173-1181.

Idris, A.B., Mohamad Roff, M.N. and Ooi, C.L. (2002). Effect of trap designs and colours on the landing rate of *Aphid gossypii,* the major insect pest of chilli in Malaysia. *OnLine Journal of Biological Sciences,* **2**, 336-339.

Ives, A.R., Kareiva, P. and Perry, R. (1993). Response of a predator to variation in prey density at three hierarchical scales: lady beetles feeding on aphids. *Ecology,* **74**, 1929-1938.

Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics,* **21**, 243-273.

James, B.D. and Luff, M.L. (1982). Cold-hardiness and development of eggs of *Rhopalosiphum insertum. Ecological Entomology,* **7**, 277-282.

Jaquiéry, J., Stoeckel, S., Nouhaud, P., Mieuzet, L., Mahéo, F., Legeai, F., Bernard, N., Bonvoisin, A., Vitalis, R. and Simon, J.C. (2012). Genome scans reveal candidate regions involved in the adaptation to host plant in the pea aphid complex. *Molecular Ecology,* **21**, 5251-5264.

Jarosik, V. and Dixon, A.F.G. (1999). Population dynamics of tree-dwelling aphid: regulation and density-independent processes. *Journal of Animal Ecology,* **68**, 726-732.

Johanowicz, D.L. and Mitchell, E.R. (2000). Effects of sweet alyssum flowers on the longevity of the parasitoid wasps *Cotesia marginiventris* (Hymenoptera: Braconidae) and *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Florida Entomologist,* **83**, 41-47.

Johnson, B. (1965). Wing polymorphism in aphids II. Interaction between aphids. *Entomologia Experimentalis et Applicata,* **8**, 49-64.

Johnson, M.T.J. (2008). Bottom-up effects on plant genotype on aphids, ants, and predators. *Ecology,* **89**, 145-154.

Jones, H.G., Hillis, R.M., Gordon, S.L. and Brennan, R.M. (2012). An approach to the determination of winter chill requirements for different *Ribes* cultivars. *Plant Biology (Stuttgard), **15***, 18-27.

Jones, M.G. (1979). Abundance of aphids on cereals from before 1973 to 1977. *Journal of Applied Ecology,* **16**, 1-22.

Kaloshian, I. (2004). Gene-for-gene disease resistance: bridging insect pest and pathogen defense. *Journal of Chemical Ecology,* **30**, 2419-2438.

Kaloshian, I., Lange, W.H. and Williamson, V.M. (1995). An aphid-resistance locus is tightly linked to the nematode-resistance gene, *Mi*, in tomato. *Proceedings of the National Academy of Sciences*, **92**, 622-625.

Kareiva, P. and Sahakian, R. (1990). Tritrophic effects of simple architectural mutation in pea plants. *Nature*, **345**, 433-434.

Karley, A.J., Douglas, A.E. and Parker, W.E. (2002). Amino acid composition and nutritional quality of potato leaf phloem sap for aphids. *Journal of Experimental Biology,* **205**, 3009-3018.

Karley, A.J., Parker, W.E., Pitchford, J.W. and Douglas, A.E. (2004). The mid-season crash in aphid populations: why and how does it occur? *Ecological Entomology,* **29**, 383-388.

Karley, A.J., Pitchford, J.W., Douglas, A.E., Parker, W.E. and Howard, J.J. (2003). The causes and processes of the mid-summer population crash of the potato aphids *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera : Aphididae). *Bulletin of Entomological Research,* **93**, 425-438.

Keep, E. and Briggs, J.B. (1971). Survey of *Ribes* species for aphid resistance. *Annals of Applied Biology,* **68**, 23-30.

Kennedy, J.S., Booth, C.O. and Kershaw, W.J.S. (1961). Host finding by aphids in the field. *Annals of Applied Biology,* **49**, 1-21.

Kenten, J. (1955). The effect of photoperiod and temperatures on reproduction in *Acyrthosiphum pisum* (Harris) and on the forms produced. *Bulletin of Entomological Research,* **46**, 599-642.

Kidd, N.A.C. and Jervis, M.A. (2007). Population dynamics. In: *Insects as natural enemies: a practical perspective,* pp. 435-503. Edited by M.A. Jervis. Dordrecht: Springer.

Kift, N.B., Mead, A., Reynolds, K., Sime, S., Barber, M.D., Denholm, I. and Tatchell, G.M. (2004). The impact of insecticide resistance in the currant-lettuce aphid, *Nasonovia ribisnigri,* on pest management in lettuce. *Agricultural and Forest Entomology,* **6**, 295-309.

Kilian, L. and Nielson, M. (1971). Differential effects of temperature on the biological activity of four biotypes of the pea aphid. *Journal of Economic Entomology,* **64**, 153-155.

Kindlmann, P. and Dixon, A.F.G. (1994). Evolution of host range in aphids. *European Journal of Entomology,* **91**, 91-96.

Kindlmann, P. and Dixon, A.F.G. (2010). Modelling population dynamics of aphids and their natural enemies. In: *Aphid biodiversity under environmental change: patterns and processes,* pp. 1-20. Edited by P. Kindlmann A.F.G. Dixon and J.P. Michaud. Dordrecht: Springer.

Kindlmann, P., Jarošík, V. and Dixon, A.F.G. (2007). Population Dynamics. In: *Aphids as Crop Pests*, pp 311-329. Edited by H.F. van Emden and R. Harrington. Wallingford: CABI Publishing.

Kleinhenz, M., Bujok, B., Fuchs, S. and Tautz, J. (2003). Hot bees in empty broodnest cells: heating from within. *The Journal of Experimental Biology,* **206**, 4217-4231.

Klueken, A.M., Hau, B., Koepke, I. and Poehling, H.-M. (2008). Comparison of techniques to survey populations of cereal aphids (Homoptera: Aphididae) in winter cereals during autumn and spring with special consideration of sample size. *Journal of Plant Diseases and Protection,* **115**, 279-287.

Knight, J.D. and Bale, J.S. (1986). Cold hardiness and overwintering of the grain aphid *Sitobion avenae*. *Ecological Entomology,* **11**, 189-197.

Koch, K.A., Potter, B.D. and Ragsdale, D.W. (2010). Non-target impacts of soybean rust fungicides on the fungal entomopathogens of soybean aphid. *Jounral of Invertebrate Pathology*, **103**, 156-164.

Kogan, M. (1994). Plant resistance in pest management. In: *Introduction to Insect Pest Management*, 3rd edn, pp. 73-128. Edited by R.L. Metcalf and W.H. Luckmann. New York: John Wiley and Sons.

Komazaki, S. (1998). Difference of egg diapause in two host races of the spirea aphid, *Aphis spiraecola*. *Entomologia Experimentalis et Applicata,* **89**, 201-205.

Krogh, A. and Zeuthen, E. (1941). The mechanism of flight preperation in some insects. *The Journal of Experimental Biology*, **18**, 1-10.

Kostál, V. (2006). Eco-physiological phases of insect diapause. *Journal of Insect Physiology,* **52**, 113-127.

Kring, J.B. (1972). Flight behaviour of aphids. *Annual Review of Entomology,* **17**, 461-492.

Kumar, A., Jain, A., Sahu, R.K., Shrivastava, M.N., Nair, S. and Mohan, M. (2005). Genetic analysis of resistance genes for the rice gall midge in two rice genotypes. *Crop Science,* **45**, 1631-1635.

Kundu, R. and Dixon, A.F.G. (1995). Evolution of complex life cycles in aphids. *Journal of Animal Ecology,* **64**, 245-255.

Kunert, G., Otto, S., Rose, U.S.R., Gershenzon, J. and Wolfgang, W.W. (2005). Alarm pheromone mediates production of winged dispersal morphs in aphids. *Ecology Letters,* **8**, 596-603.

Kuo, M.H., Chiu, M.C. and Perng, J.J. (2006). Temperature effects on life history traits of the corn leaf aphid, *Rhopalosiphum maidis* (Homoptera: Aphididae) on corn in Taiwan. *Applied Entomology and Zoology*, **41**, 171-177.

Lamb, K.P. (1959). Composition of the honeydew of the aphid *Brevicoryne brassicae* (L.) feeding on swedes (*Brassica napobrassica* DC.). *Journal of Insect Physiology*, **3**, 1-13.

Lamb, K.P. (1961). Some effects of fluctuating temperatures on metabolism, development, and rate of population growth in the cabbage aphid, *Brevicoryne brassicae*. *Ecology,* **42**, 740-745.

Lamb, R. and Mackay, P.A. (1988). Effects of temperature on the development rate and adult weight of Australian populations of *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). *Memoirs of the Entomological Society of Canada,* **120**, 49-55.

Lamb, R.J. and Pointing, P.J. (1972). Sexual morph determination in the pea aphid, *Acyrthosiphon pisum. Journal of Insect Physiology,* **18**, 2029-2042.

Lamb, R.J. and Pointing, P.J. (1975). The reproductive sequence and sex determination in the aphid, *Acyrthosiphon pisum. Journal of Insect Physiology,* **21**, 1443-1446.

Lamb, R.J., MacKay, P.A. and Gerber, G.H. (1987). Are development and growth of peas aphids, *Acyrthosiphon pisum*, in North America adapted to local temperatures? *Oecologia,* **72**, 170-177.

Hille Ris Lambers, D. (1966). Polymorphism in Aphididae. *Annual Review of Entomology,* **11**, 47-78.

Landis, D.A., Wratten, S.D. and Gurr, G.M. (2000). Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology,* **45**, 175-201.

Lang, A. (2003). Intraguild interference and biocontrol effects of generalist predators in a winter wheat field. *Oecologia*, **134**, 144-153.

Latteur, G. and Jansen, J.-P. (2002). Effects of 20 fungicides on the infectivity of conidia of the aphid entomopathogenic fungus *Erynia neoaphidis. BioControl*, **47**, 435-444.

Le Ralec, A., Anselme, C., Outreman, Y., Poirié, M., van Baaren, J., Le Lann, C. and van Alphen, J.J. (2010). Evolutionary ecology of the interactions between aphids and their parasitoids. *Comptes Rendus Biologies,* **333**, 554-565.

Le Roux, V., Dugravot, S., Campan, E., Dubois, F., Vincent, C. and Giordanengo, P. (2008). Wild Solanum resistance to aphid: antixenosis or antibiosis? *Journal of Economic Entomology*, **101**, 584-591.

Leather, S.R. (1980). Egg survival in the bird cherry-oat aphid, *Rhopalosiphum padi. Entomologia Experimentalis et Applicata,* **27**, 96-97.

Leather, S.R. (1981). Factors affecting egg survival in the bird cherry-oat aphid, *Rhopalosiphum padi. Entomologia Experimentalis et Applicata,* **30**, 197-199.

Leather, S.R. and Dixon, A.F.G. (1984). Aphid growth and reproductive rates. *Entomologia Experimentalis et Applicata,* **35**, 137-140.

Leather, S.R. and Owuor, A. (1996). The influence of natural enemies and migration on spring populations of the green spruce aphid, *Elatobium abietinum* Walker (Hom., Aphididae). *Journal of Applied Entomology,* **120**, 529-536.

Leather, S.R., Walters, K.F.A. and Bale, J.S. (1993). *The ecology of insect overwintering*. New York: Cambridge University Press.

Lees, A.D. (1959). The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid *Megoura viciae* Buckton-I: The influence of these factors on apterous virginoparae and their progeny. *Journal of Insect Physiology*, **3**, 92-117.

Lees, A.D. (1963). The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid *Megoura viciae* Buckton—III. Further properties of the maternal switching mechanism in apterous aphids. *Journal of Insect Physiology*, **9**, 153–164.

Lees, A.D. (1967). The production of apterous and alate forms in the aphid *Megoura viciae* Buckton, with special reference to the role of crowding. *Journal of Insect Physiology*, **13**, 289-318.

Lees, A.D. (1973). Photoperiodic time measurement in the aphid *Megoura viciae. Journal of Insect Physiology*, **19**, 2279-2316.

Lees, A.D. (1986). Some effects of temperature on the hour glass photoperiod timer in the aphid *Megoura viciae. Journal of Insect Physiology*, **32**, 79-89.

Levins, R. and MacArthur, R. (1969). An hypothesis to explain the incidence of monophagy. *Ecology*, **50**, 910-911.

Lindqvist, K. (1960). Cytogenetic studies in the *Serriola* group *Lactuca. Hereditas*, **46**, 75-151.

Liu, D. (1994). Production of alate in response to low temperature in aphids: a trait of seasonal adaptation. In: *Insect life-cycle polymorphism: theory, evolution and ecological Consequences for seasonality and diapause control*, pp. 245-261. Edited by H.V. Danks. Dordrecht: Kluwer Academic Publishers.

Liu, S. and Meng, X. (1999). Modelling development time of *Myzus persicae* (Hemiptera: Aphididae) at constant and natural temperatures. *Bulletin of Entomological Research*, **89**, 53-63.

Liu, Y.B. (2004). Distribution and population development of *Nasonovia ribisnigri* (Homoptera: Aphididae) in iceberg lettuce. *Journal of Economic Entomology*, **97**, 883-890.

Liu, Y.B. (2005). Ultralow oxygen treatment for postharvest control of *Nasonovia ribisnigri* (Homoptera: Aphididae) on iceberg lettuce. *Journal of Economic Entomology*, **98**, 1899-1904.

Liu, Y.B. and McCreight, J.D. (2006). Responses of *Nasonovia ribisnigri* (Homoptera : Aphididae) to susceptible and resistant lettuce. *Journal of Economic Entomology*, **99**, 972-978.

Lodge, J., Lund, P. and Minchin, S. (2007). *Gene cloning: principles and applications*. Abington: Taylor and Francis Group.

Lombaert, E., Carletto, J., Piotte, C., Fauvergue, X., Lecoq, H., Vanlerberghe-Masutti, F. and Lapchin, L. (2009). Response of the melon aphid, *Aphis gossypii*, to host-plant resistance: evidence for high adaptive potential despite low genetic variability. *Entomologia Experimentalis et Applicata,* **133**, 46–56.

Losey, J.E. and Denno, R.F. (1999). Factors facilitating synergistic predation: the central role of synchrony. *Ecological Applications,* **9**, 378-386.

Lowe, A.D. (1966). Processes within pasture and crop ecosystems: Some effects of weather on aphids in crops and pastures in Canterbury, New Zealand. *New Zealand Journal of Ecology,* **13**, 86-90.

Lowe, H.J. (1974). Intraspecific variation of *Myzus persicae* on sugar beet (*Beta vulgaris*). *Annals of Applied Biology*, **78**, 15-26.

Loxdale, H.D. (2008). The nature and reality of the aphid clone: genetic variation, adaptation and evolution. *Agricultural and Forest Entomology,* **10**, 81-90.

Loxdale, H.D. (2009). What's in a Clone: The Rapid Evolution of Aphid Asexual Lineages in Relation to Geography, Host Plant Adaptation and Resistance to Pesticides. In: *Lost Sex- The Evolutionary Biology of Parthenogenesis*, pp. 535-557. Edited by I. Schön, K. Martens and P. van Dijk. Netherlands: Springer.

Loxdale, H.D. and Lushai, G. (2003). Rapid changes in clonal lines: the death of a 'sacred cow'. *Biological Journal of the Linnean Society*, **79**, 3-16.

Loxdale, H.D. and Lushai, G. (2007). Population Genetic Issues: the unfolding story using molecular markers. In: *Aphids as Crop Pests*, pp. 31-67. Edited by H.F. van Emden and R. Harrington. Wallingford: CABI Publishing.

Luck, R.F., Shepard, B.M. and Kenmore, P.E. (1988). Experimental methods for evaluating arthropod natural enemies. *Annual Review of Entomology,* **33**, 367-389.

Lushai, G., Hardie, J. and Harrington, R. (1996). Dipause termination and egg hatch in the bird cherry aphid, *Rhapalosiphum padi*. *Entomologia Experimentalis et Applicata,* **81**, 113-115.

Lushai, G., De Barro, P.J., David, O., Sherratt, T.N. and Maclean, N. (1998). Genetic variation within a parthenogentic lineage. *Insect Molecular Biology,* **7**, 337-334.

Lushai, G., Loxdale, H.D., Brookes, C.P., von Mende, N., Harrington, R. and Hardie, J. (1997). Genotypic variation among different phenotypes within

aphid clones. *Proceedings of the Royal Society B: Biological Sciences,* **264,** 725-730.

MacKay, P.A. (1987). Production of sexual and asexual morphs and changes in reproductive sequence associated with photoperiod in the pea aphid, *Acyrthosiphon pisum* (Harris). *Canadian Journal of Zoology,* **65**, 2602-2606.

MacKay, P.A. and Wellington, W.G. (1977). Maternal age as a source of variation in the ability of an aphid to produce dispersing forms. *Researches on Population Ecology,* **18**, 195-209.

Mackenzie, A. (1996). A trade-off for host plant utilization in the black bean aphid *Aphis fabae. Evolution,* **50**, 155-162.

Mackenzie, J.R. and Vernon, R.S. (1988). Sampling for distribution of the lettuce aphid, *Nasonovia ribisnigri* (Homoptera: Aphididae) in field and within heads. *Journal of the Entomological Society of British Columbia,* **85**, 10-14.

Malloch, G., Highet, F., Kasprowicz, L., Pickup, J., Neilson, R. and Fenton, B. (2006). Microsatellite marker analysis of peach-potato aphids (*Myzus persicae*, Homoptera: Aphididae) from Scottish suction traps. *Bulletin of Entomological Research,* **96**, 573-582.

Mann, J.A., Tatchell, G.M., Dupuch, M.J., Harrington, R., Clark, S.J. and McCartney, H.A. (1995). Movement of apterous *Sitobion avenae* (Homoptera: Aphididae) in response to leaf disturbances caused by wind or rain. *Annals of Applied Biology,* **126**, 417-427.

Margaritopoulos, J.T., Tsitsipis, J.A., Goudoudaki, S. and Blackman, R.L. (2002). Life cycle variation of *Myzus persicae* (Hemiptera: Aphididae) in Greece. *Bulletin of Entomological Research,* **92**, 309-319.

Mashanova, A., Gange, A.C. and Jansen, V.A.A. (2008). Density-dependent dispersal may explain the mid-season crash in some aphid populations. *Population Ecology,* **50**, 285-292.

Matsuka, M. and Mittler, T.E. (1979). Production of males and gynoparae by apterous vivparae of *Myzus persicae* continously exposed to different scotoperiods. *Journal of Insect Physiology,* **25**, 587-593.

Matsumoto, K., Sawada, H., Matsumoto, K., Hamada, H., Yoshimoto, E., Ito, T., Takeuchi, S., Tsuda, S., Suzuki, K., Kaboyashi, K., Kiba, A., Okuno, T. and Hikichi, Y. (2008). The coat protein gene of tobamovirus P_0 pathotype is a determinant for activation of temperature-insensitive L^{1a} -gene mediated resistance in Capsicum plants. *Archives of Virology,* **153**, 645-650.

Mattson, W.J. and Haack, R.A. (1987). Role of drought in outbreaks of plant-eating insects. *Bioscience*, **37**, 110-118.

McCreight, J.D. (2008). Potential sources of genetic resistance in *Lactuca* spp. the lettuce aphid *Nasanovia ribisnigri* (Mosely) (Homoptera : Aphididae). *Hortscience,* **43**, 1355-1358.

McCreight, J.D. and Liu, Y-B. (2012). Resistance to lettuce aphid (*Nasonovia ribisnigri*) Biotype 0 in wild lettuce accessions PI 491093 and PI 274378. *HortScience,* **47**, 179-184.

McDougall, S., Westcott, A., Baker, G. and Santo, P.D. (2004). *Lettuce aphid threat.* [Online]. Horticulture Australia. http://www.vgavic.org.au/pdf/VegeNote-Lettuce-Aphid.pdf [Accessed: 21 January 2011].

McLeod, D.G.R. and Beck, S.D. (1963). Photoperiodic termination of diapause in an insect. *Biological Bulletin,* **124**, 84-96.

McLeod, P.J., Steinkraus, D.C., Correll, J.C. and Morelock, T.E. (1998). Prevalence of *Erynia neoaphidis* (Entomophthorales: Entomophthoraceae) infections of green peach aphid (Homoptera: Aphididae) on spinach in Arkansas River Valley. *Environmental Entomology,* **27**, 796-800.

McMaster, G.S. and Wilhelm, W.W. (1997). Growing degree-days: one equation, two interpretations. *Agricultural and Forest Meteorology,* **87**, 291-300.

McVean, R.I.K., Dixon, A.F.G. and Harrington, R. (1999). Causes of regional and yearly variation in pea aphid numbers in eastern England. *Journal of Applied Entomology,* **123**, 495-502.

Meihls, L.N., Clark, T.L., Bailey, W.C. and Ellersieck, M.R. (2010). Population growth of soybean aphid, *Aphis glycines*, under varying levels of predator exclusion. *Journal of Insect Science,* **10**, 1-18.

Mellanby, K. (1939). Low temperature and insect activity. *Proceedings of the Royal Society B: Biological Sciences,* **127**, 473-487.

Meng, F., Han, Y., Tend, W., Li, Y. and Li, W. (2011). QTL underlying the resistance to soybean aphid (*Aphis glycines* Matsumura) through isoflavone-mediated antibiosis in soybean cultivar 'Zhongdou 27'. *Theoretical and Applied Genetics,* **123**, 1459-1465.

Messina, F.J. (1993). Winter mortality of the Russian wheat aphid (Homoptera: Aphididae) on range grasses in Northern Utah. *Great Basin Naturalist,* **53**, 310-313.

METOffice. (2012). *Winter 2011/12*. [Online]. MET Office <http://www.metoffice.gov.uk/climate/uk/2012/winter.html> [Accessed: 21 November 2012)

Mills, N.J. and Latham, D.R. (2009). Quantifying the role of predation in the seasonal dynamics of aphid populations. *Redia,* **92**, 153-157.

Moran, N.A. (1983). Seasonal shifts in host usage in *Uroleucon gravicorne* (Homoptera: Aphididae) and implications for the evolution of host alternation in aphids. *Ecological Entomology*, **8**, 371-382.

Moran, N.A. (1988). The Evolution of host-plant alternation in aphids: evidence for specialization as a dead end. *The American Naturalist*, **132**, 681-706.

Moran, N.A. (1992). The evolution of aphid life cycles. *Annual Review of Entomology*, **37**, 321-348.

Moreno, A., Bertolini, E., Olmos, A., Cambra, M. and Fereres, A. (2007). Estimation of vector propensity for Lettuce mosaic virus based on viral detection in single aphids. *Spanish Journal of Agricultural Research*, **5**, 376-384.

Müller, C.B., Williams, I.S. and Hardie, J. (2001). The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecological Entomology*, **26**, 330-340.

Müller, C.B., Adriaanse, I.C.T., Belshaw, R. and Godfray, H.C.J. (1999). The structure of an aphid-parasitoid community. *Journal of Animal Ecology*, **68**, 346-370.

Nam, K.J. and Hardie, J. (2012). Host acceptance by aphids: probing and larviposition behaviour of the bird cherry-oat aphid, *Rhopalosiphum padi* on host and non host-plants. *Journal of Insect Physiology*, **58**, 660-668.

Nebreda, M., Michelena, J.M. and Fereres, A. (2005). Seasonal abundance of aphid species on lettuce crops in Central Spain and identification of their main parasitoids. *Journal of Plant Diseases and Protection*, **112**, 405–415.

Nebreda, M., Moreno, A., Pérez, N., Palacios, I., Seco-Fernández, V. and Fereres, A. (2004). Activity of aphids associated with lettuce and broccoli in Spain and their efficiency as vectors of Lettuce mosaic virus. *Virus Research*, **100**, 83-88.

Neven, L.G. (2000). Physiological responses of insects to heat. *Postharvest Biology and Technology*, **21**, 103-111.

Newton, C. and Dixon, A.F.G. (1988). The cost of switching from asexual to sexual reproduction in an aphid. *Entomologia Experimentalis et Applicata*, **47**, 283-287.

Newton, R.H. and Dixon, A.F.G. (1987). Methods of hatching the eggs and rearing fundatrices of the English grain aphid, *Sitobion avenae*. *Entomologica Experimentalis et Applicata*, **45**, 277-281.

Nielsen, C. and Hajek, A.E. (2005). Control of invasive Soybean Aphid, *Aphis glycines* (Hemiptera: Aphididae), populations by existing natural enemies in

New York state, with emphasis on entomopathogenic fungi. *Environmental Entomology,* **34**, 1036-1047.

Niemeyer, H.M. (1991). Secondary plant chemicals in aphid-host interactions. In: *Aphid-plant interactions: populations to molecules*, pp. 101-111. Edited by D.C. Peters, J.A. Webster and C.S. Chlouber. Oklahoma: Oklahoma Agricultural Experiment Station.

Nomikou, M., Janssen, A. and Sabelis, M.W. (2003). Herbivore host plant selection: whitefly learns to avoid host plants that harbour predators of her offspring. *Oecologia,* **136**, 484-488.

Nunnenmacher, L. and Goldbach, H.E. (1996). Aphids on lettuce: the effects of excluding aphid predators. *Bulletin IOBC/WPRS,* **19**, 38-47.

Obrycki, J.J. and Kring, T.J. (1998). Predaceous Coccinellidae in biological control. *Annual Review of Entomology*, **43**, 295-321.

Ochieng-Odero, J.P.R. (1991). The effect of photoperiod and thermophotoperiod on the larval critical weight, latent feeding period, larval maximum weight and fecundity of *Cnephasia jactatana* (Walker) (Lepidoptera: Tortricidae). *Journal of Insect Physiology*, **37**, 441-445.

Oswald, C.J. and Brewer, M.J. (1997). Aphid-barley interactions mediated by water stress and barley resistance to Russian wheat aphid (Homoptera: Aphididae). *Environmental Entomology,* **26**, 591-602.

Palumbo, J. (2000a). *Population and distribution of aphid species on head lettuce in the Yuma valley.* University of Arizona, College of Agriculture and Life Sciences. 2000 Vegetable Report.

Palumbo, J.C. (2000b). *Seasonal abundance and control of the lettuce aphid, Nasonovia ribisnigri, on head lettuce in Arizona.* University of Arizona, College of Agriculture and Life Sciences. 2000 Vegetable Report.

Palumbo, J.C. (2002). *Population Growth of Lettuce Aphid, Nasonovia ribisnigri, on Resistant Butter and Head Lettuce Cultivars*. University of Arizona, College of Agriculture and Life Sciences. 2002 Vegetable Report.

Parker, W.E., Collier, R.H., Ellis, P.R., Mead, A., Chandler, D., Smyth, J.A.B. and Tatchell, G.M. (2002). Matching control options to a pest complex: the integrated pest management of aphids in sequentially-planted crops of outdoor lettuce. *Crop Protection,* **21**, 235-248.

Peccoud, J., Simon, J.E., von Dohlen, C., Coeur d'acier, A., Plantegenest, M., Vanlerberghe-Masutti, F. and Jousselin, E. (2010). Evolutionary history of aphid-plant associations and their role in aphid diversification. *Comptes Rendus Biologies,* **333**, 474-487.

Pelletier, Y., Pompon, J., Dexter, P. and Quiring, D.T. (2010). Biological performance of *Myzus persicae* and *Macrosiphum euphorbiae* (Homoptera: Aphididae) on seven wild *Solanum* species. *Annals of Applied Biology,* **156**, 329-336.

Petterson, J., Tjallingii, W.D. and Hardie, J. (2007). Host-plant Selection and Feeding. In: *Aphids as Crop Pests*, pp. 87-113. Edited by H.F. van Emden and R. Harrington. Wallingford: CABI Publishing.

Phelan, P.L., Montgomery, M.E. and Nault, L.R. (1976). Orientation and locomotion of apterous aphids dislodged from their hosts by alarm pheromone. *Annals of the Entomological Society of America*, **69**, 1153-1156.

Phelps, K., Collier, R.H., Reader, R.J. and Finch, S. (1993). Monte Carlo simulation method for forecasting the timing of pest insect attacks. *Crop Protection,* **12**, 335-342.

Phillips, S.W., Bale, J.S. and Tatchell, G.M. (1999). Escaping an ecological dead-end: asexual overwintering and morph determination in the lettuce root aphid *Pemphigus bursarius* L. *Ecological Entomology*, **24**, 336-344.

Phillips, S.W., Bale, J.S. and Tatchell, G.M. (2000). Overwintering adaptations in the lettuce root aphid *Pemphigus bursarius* (L.). *Journal of Insect Physiology,* **46**, 353-363.

Pickett, A., Wadhams, L.J., Woodcock, C.M. and Hardie, J. (1992). The chemical ecology of aphids. *Annual Review of Entomology,* **37**, 67-90.

Plantegenest, M., Pierre, J.S., Dedryver, C.A. and Kindlmann, P. (2001). Assessment of the relative impact of different natural enemies on population dynamics of the grain aphid *Sitobion avenae* in the field. *Ecological Entomology,* **26**, 404–410.

Pons, X., Comas, J. and Albajes, R. (1995). Occurrence of holocyclic and anholocyclic populations of *Rhopalosiphum padi* and *Sitobion avenae* (Hom., Aphididae) in the northeast of Spain. *Journal of Applied Entomology,* **119**, 171-175.

Powell, G. and Hardie, J. (2000). Host-selection behaviour by genetically identical aphids with different plant preferences. *Physiological Entomology*, **25**, 54-62.

Powell, G., Tosh, C.R. and Hardie, J. (2006). Host plant selection by aphids: behavioural, evolutionary, and applied perspectives. *Annual Review of Entomology*, **51**, 309-330.

Powell, G., Maniar, S.P., Pickett, J.A. and Hardie, J. (1999). Aphid responses to non-host epicuticular lipids. *Entomologia Experimentalis et Applicata,* **91**, 115-123.

Powell, S.J. and Bale, J.S. (2004). Cold shock injury and ecological costs of rapid cold hardening in the grain aphid *Sitobion avenae* (Hemiptera: Aphididae). *Journal of Insect Physiology*, **50**, 277-284.

Powell, S.J. and Bale, J.S. (2008). Intergenerational acclimation in aphid overwintering. *Ecological Entomology*, **33**, 95-100.

Powell, W. and Pell, J.K. (2007). Biological Control. In: *Aphids as Crop Pests*, pp. 469-513. Edited by H.F. van Emden and R. Harrington. Wallingford: CABI Publishing.

Prasanna, B.M. (2007). *QTL mapping and its application in crop plants*. [Online] Indian Agricultural Statistics Research Institute. <http://www.iasri.res.in/ebook/EB_SMAR/index.htm> [Accessed: 27 November 2011]

Prosser, W.A. and Douglas, A.E. (1992). A test of the hypotheses that nitrogen is upgraded and recycled in an aphid (*Acyrthosiphon pisum*) symbiosis. *Journal of Insect Physiology*, **38**, 93-99.

PSD. (2003). *Food and environment protection act 1985 control of pesticide regulations 1986 (S.I. 1986 NO. 1510): Approval for off-label use of an approved pesticide product.* Notice of approval number: 1886 of 2003. Pesticide Safety Directorate.

Puterka, G.J. and Slosser, J.E. (1986). Influence of host and temperature on greenbug, *Schizaphis graminum* (Rondani), egg hatch. *Southwestern Entomologist*, **11**, 75-81.

Ragsdale, D.W., McCornack, B.P., Venette, R.C., Potter, B.D., MacRae, I.V., Hodgson, E.W., O'neal, M.E., Johnson, K.D., O'neil, R.J., DiFonzo, C.D., Hunt, T.E., Glogoza, P.A. and Cullen, E.M. (2007). Economic threshold for soybean aphid (Hemiptera: Aphididae). *Journal of Economic Entomology* **100**, 1258-1267.

Rausher, M.D. (1984). Tradeoffs in performance on different hosts: evidence from within- and between-site variation in beetle *Deloyala guttata*. *Evolution* **38**, 582-595.

Reese, J.C., Schwenke, J.R., Lamont, P.S. and Zehr, D.D. (1994). Importance and quantification of plant tolerance in crop pest management programs for aphids: greenbug resistance in sorghum. *Journal of Agricultural Entomology*, **11**, 255-270.

Reinink, K. and Dieleman, F.L. (1989). Comparison of sources of resistance to leaf aphids in lettuce (*Lactuca sativa L.*). *Euphytica*, **40**, 21-29.

Rekika, D., Stewart, K.A., Boivin, G. and Jenni, S. (2009). Row covers reduce insect populations and damage and improve early season crisphead lettuce production. *International Journal of Vegetable Science*, **15**, 71-82.

Reynolds, P.G. and Cuddington, K. (2012). Effects of plant gross morphology on predator consumption rates. *Environmental Entomology*, **41**, 508-515.

RIS. (No date-a). *Currant-Lettuce Aphid- Nasonovia ribisnigri*. [Online]. Rothamsted Insect Survey. <http://www.rothamsted.ac.uk/insect-survey/STNasonovia_ribisnigri.php> [Accessed: 30 April 2012].

RIS. (No date-b). *Aphid bulletin*. [Online]. Rothamsted Insect Survey. <http://www.rothamsted.ac.uk/insect-survey/STAphidBulletin.php> [Accessed: 20 January 2013].

Rispe, C. and Pierre, J.S. (1998). Coexistence between cyclical parthenogens, obligate parthenogens, and intermediates in a fluctuating environment. *Journal of Theoretical Biology*, **195**, 97-110.

Robert, Y. (1987). Aphids and their environment; Dispersion and migration. In: *Aphids; their biology, natural enemies and control, World Crop Pests, Vol. 2a,* pp. 299-313. Edited by A.K. Minks and P. Harrewijn. Amsterdam: Elsevier.

Rohitha, B.H. and Penman, D.R. (1983). Effect of temperature on biology of bluegreen lucerne aphid, *Acyrthosiphon kondoi*. *New Zealand Journal of Zoology*, **10**, 299-308.

Roltsch, W.J., Zalom, F.G., Strawn, A.J., Strand, J.F. and Pitcairn, M.J. (1999). Evaluation of several degree-day estimation methods in California climates. *International Journal of Biometeorology*, **42**, 169-176.

Rotheray, G.E. (1989). *Aphid predators*. Slough: Richmond Publishing.

Rufingier, C., Pasteur, N., Lagnel, J., Martin, C. and Navajas, M. (1999). Mechanisms of insecticide resistance in the aphid *Nasonovia ribisnigri* (Mosley) (Homoptera : Aphididae) from France. *Insect Biochemistry and Molecular Biology*, **29**, 385-391.

Rutledge, C.E., O'Neil, R.J., Fox, T.B. and Landis, D.A. (2004). Soybean aphid predators and their use in Integrated Pest Management. *Annals of the Entomological Society of America*, **97**, 240-248.

Sandström, J. and Pettersson, J. (1994). Amino acid composition of phloem sap and the relation to intraspecific variation in pea aphid (*Acyrthosiphon pisum*) performance. *Journal of Insect Physiology*, **40**, 947-955.

Sandström, J., Telang, A. and Moran, N.A. (2000). Nutritional enhancement of host plants by aphids - a comparison of three aphid species on grasses. *Journal of Insect Physiology*, **46**, 33-40.

Satar, S., Kersting, U. and Uygun, N. (2008). Effect of temperature on population parameters of *Aphis gossypii* Glover and *Myzus persicae* (Sulzer)

(Homoptera: Aphididae) on pepper. *Journal of Plant Diseases and Protection,* **115**, 69-74.

Sauge, MH., Lambert, P. and Pascal, T. (2012). Co-localisation of host plant resistance QTLs affecting the performance and feeding behaviour of the aphid *Myzus persicae* in the peach tree. *Heredity,* **108**, 292-301.

Schmidt, M.H., Thewes, U., Thies, C. and Tscharntke, T. (2004). Aphid suppression by natural enemies in mulched cereals. *Entomologia Experimentalis et Applicata,* **113**, 87-93.

Schmidt, N.P., O'neal, M.E. and Dixon, P.M. (2008). Aphidophagous predators in Iowa Soybean: a community comparison across multiple years and sampling methods. *Annals of the Entomological Society of America,* **101**, 341-350.

Schoonhoven, L.M. and Derksen-Koppers, I. (1976). Effects of some allelochemics on food uptake and survival of a polyphagous aphid, *Myzus persicae. Entomologia Experimentalis et Applicata,* **19**, 52-56.

Schoonhoven, L.M., van Loon, J.J.A. and Dicke, M. (2005). *Insect-Plant Biology.* 2nd edn. New York: Oxford University Press.

Schowalter, T.D. (2006). Population dynamics. In: *Insect Ecology: An Ecosystem Approach*. 2nd, pp. 576. Edited by T.D. Schowalter. London: Academic Press.

Schädler, M., Brandl, R. and Kempel, A. (2010). Host plant genotype determines bottom-up effects in an aphid-parasitoid-predator system. *Entomologia Experimentalis et Applicata,* **135**, 162-169.

Shingleton, A.W., Sisk, G.C. and Stern, D.L. (2003). Diapause in the pea aphid (*Acyrthosiphon pisum*) is a slowing but not a cessation of development. *BMC Developmental Biology,* **3,** 1-12.

Shu-sheng, L. and Hughes, R.D. (1987). The Influence of temperature and photoperiod on the development, survival and reproduction of the sowthistle aphid, *Hyperomyzus lactucae. Entomologia Experimentalis et Applicata,* **43**, 31-38.

Simon, J.C., Rispe, C. and Sunnucks, P. (2002). Ecology and evolution of sex in aphids. *Trends in Ecology and Evolution,* **17**, 34-39.

Simon, J.C., Baumann, S., Sunnucks, P., Hebert, P.D., Pierre, J.S., Le Gallic, J.F. and Dedryver, C.A. (1999). Reproductive mode and population genetic structure of the cereal aphid *Sitobion avenae* studied using phenotypic and microsatellite markers. *Molecular Ecology,* **8**, 531-545.

Singh, D.P. and Singh, A. (2005). *Disease and insect resistance in plants*. Enfield: Science Publishers.

Skirvin, D.J., Perry, J.N. and Harrington, R. (1997). The effect of climate change on an aphid-coccinellid interaction. *Global Change Biology*, **3**, 1-11.

Skirvin, D.J., Kravar-Garde, L., Reynolds, K., Wright, C. and Mead, A. (2011). The effect of within-crop habitat manipulations on the conservation biological control of aphids in field-grown lettuce. *Bulletin of Entomological Research*, **101**, 623-631.

Slade, A.J. and Knauf, V.C. (2005). TILLING moves beyond functional genomics into crop improvement. *Transgenic Research*, **14**, 109-115.

Smilde, W.D., Ludlage, R., Deinum, D. and van Houwelingen, M. (2009). First report of a resistance breaking biotype of the lettuce leaf aphid *Nasonovia ribisnigri* (Unpublished). The Netherlands: Naktuinbouw.

Smiley, J. (1978). Plant chemistry and the evolution of host specificity: new evidence from *Heliconius* and *Passiflora*. *Science*, **201**, 745-747.

Smith, C.M. and Boyko, E.V. (2007). The molecular bases of plant resistance and defense responses to aphid feeding: current status. *Entomologia Experimentalis et Applicata*, **122**, 1-16.

Smith, H.A. and Chaney, W.E. (2007). A survery of syrphid predators of *Nasonovia ribisnigri* in organic lettuce on the central coast of California. *Journal of Economic Entomology*, **100**, 39-48.

Smith, H.A., Chaney, W.E. and Bensen, T.A. (2008). Role of syrphid larvae and other predators in suppressing aphid infestations in organic lettuce on California's central coast. *Journal of Economic Entomology*, **101**, 1526-1532.

Smith, J.M. (1971). What use is sex? *Journal of Theoretical Biology*, **30**, 319-335.

Snyder, W.E. and Ives, A.R. (2001). Generalist predators disrupt biological control by a specialist parasitoid. *Ecology*, **82**, 705-716.

Steinkraus, D.C. (2006). Factors affecting transmission of fungal pathogens of aphids. *Journal of Invertebrate Pathology*, **92**, 125-131.

Stoker, H.S. (2009). Enzymes and Vitamins. In: *General, Organic, And Biological Chemistry*, 5th Edn, p. 960. Edited by H.S. Stoker. Belmont: Brooks/Cole.

Strathdee, A.T., Howling, G.G. and Bale, J.S. (1995). Cold hardiness of overwintering aphid eggs. *Journal of Insect Physiology*, **41**, 653-657.

Stufkens, M.A.W. and Teulon, D.A.J. (2003). Distributionm, host range and flight pattern of the lettuce aphid in New Zealand. *New Zealand Plant Protection*, **56**, 27-32.

Sutherland, O.R.W. (1969). The role of crowding in the production of winged forms by two strains of the pea aphid, *Acrthosiphon pisum. Journal of Insect Physiology*, **15**, 1385-1410.

Tamaki, G. and Long, G.E. (1978). Predator complex of the green peach aphid on sugarbeets: expansion of the predator power and efficacy model. *Environmental Biology*, **7**, 835-842.

Tamaki, G. and Weeks, R.E. (1973). The impact of predators on populations of green peach aphids on field-grown sugarbeets. *Environmental Entomology*, **2**, 345-349.

Tatchell, M. (2004). Guide to the identification of aphids found commonly on Lettuce, Brassicas and Potatoes (Unpublished).

Tatchell, M., Ellis, B., Collier, R., Chandler, D., Mead, A., Wadhams, L., Parker, B., Blood-Smyth, J. and Vice, L. (1998). *Integrated pest management of aphids on outdoor lettuce crops*. Horticultural Development Company. Final Report Project Number FV162.

Tauber, M.J. and Tauber, C.A. (1972). Geographic variation in critical photoperiod and in diapause intensity of *Chrysopa carnea* (Neuroptera). *Journal of Insect Physiology*, **18**, 25-29.

Tauber, M.J. and Tauber, C.A. (1976). Insect seasonality - diapause maintenance, termination, and postdiapause development. *Annual Review of Entomology*, **21**, 81-107.

Tauber, M.J., Tauber, C.A. and Masaki, S. (1986). *Seasonal adaptations of insects*. New York: Oxford University Press.

Taylor, L.R. (1963). Analysis of the Effect of Temperature on Insects in Flight. *Journal of Animal Ecology*, **32**, 99-117.

Taylor, L.R. (1977). Migration and the spatial dynamics of an aphid, *Myzus persicae. Journal of Animal Ecology*, **46**, 411-423.

Teekens, K.C., Thabuis, A.P.P. and van Herwijnen, Z.O. (2010). Lettuce that is resistant to the lettuce aphid *Nasonovia ribisnigri* Biotype 1. Netherlands. Patent number WO2011058192. Filing date 16 November 2010. Publication date 19 May 2011.

Telang, A., Sandström, J., Dyreson, E. and Moran, N.A. (1999). Feeding damage by *Diuraphis noxia* results in a nutritionally enhanced phloem diet. *Entomologia Experimentalis et Applicata*, **91**, 403-412.

ten Broeke, C.J.M., van Loon, J.J.A. and Dicke, M. (2010). The resistance mechanism of lettuce against aphids. *Abstract for Technology Top Institute-Green Genetics Meeting*, Utrecht, 22 September 2010.

ten Broeke, C.J.M., van Loon, J.J.A. and Dicke, M. (2011). Feeding behaviour and perfomance studies on different populations of the black-currant aphid *Nasonovia ribisnigri* on resistant and susceptible lettuce cultivars. *Proceedings of the 2011 Hemipteran-Plant International Symposium*. Piracicaba, Brazil, 11-14 July 2011, p. 4.

Tenhumberg, B. and Poehling, H.-M. (1995). Syrphids as natural enemies of cereal aphids in Germany: aspects of their biology and efficacy in different years and regions. *Agriculture, Ecosystems and Environment,* **52**, 39-43.

Thomas, S.M., Obermayr, U., Fischer, D., Kreyling, J. and Beierkuhnlein, C. (2012). Low-temperature threshold for egg survival of a post-diapause and non-diapause European aedine strain, *Aedes albopictus* (Diptera: Culicidae). *Parasites and Vectors,* **5**:100.

Timeanddate. (2010). *Sunrise and Sunset.* [Online] Timeanddate <http://www.timeanddate.com> [Accessed: 02 December 2009].

Tjallingii, W.F. and Esch, TH. H. (1993). Fine-structure of aphids stylet routes in plant-tissues in correlation with EPG signals *Physiological Entomology,* **18**, 317-328.

Tofangsazi, N., Kheradmand, K., Shahrokhi, S. and Talebi, A.A. (2010). Temperature-dependent life history of *Schizaphis graminum* on barley. *Bulletin of Insectology,* **63**, 79-84.

Tosh, C.R., Powell, G. and Hardie, J. (2002). Maternal reproductive decisions are independent of feeding in the black bean aphid, *Aphis fabae. Journal of Insect Physiology,* **48**, 619-629.

Trumble, J.T. (1982). Within-plant distribution and sampling of aphids (Homoptera: Aphididae) in Broccoli in South California. *Journal of Economic Entomology,* **75**, 587-592.

UCIPM. (2003). *Degree-Days*. [Online]. Agriculture and Natural Resources, University of California http://www.ipm.ucdavis.edu/WEATHER/ddconcepts.html> [Accessed: 23 January 2013].

van der Arend, A.J.M. (2003). The possibility of *Nasonovia ribisnigri* resistance breaking biotype developmen due to plant host resistance: a literature study. *Eucarpia Leafy Vegetables Conference 2003.* Noordwijkerhout, The Netherlands, 19-21 March 2003, p. 75-81.

van Helden, M. (1993). The chemical basis of resistance to *Nasonovia ribisnigri* (Aphididae) in lettuce. IOBC/WPRS *Bulletin OILB SROP,* **16**, 69-76.

van Helden, M. and Tjallingii, W.F. (1993). Tissue localisation of lettuce resistance to the aphid *Nasonovia ribisnigri* using electrical penetration graphs. *Entomologia Experimentalis et Applicata,* **68**, 269-278.

van Helden, M., Tjallingii, W.F. and Dieleman, F.L. (1993). The resistance of lettuce (*Lactuca sativa L.*) the the *Nasonovia ribisnigri*: bionomics of *N. ribisnigri* on near isogenic lettuce lines. *Entomologia Experimentalis et Applicata,* **66**, 53-58.

van Helden, M., Tjallingii, W.F. and van Beek, T.A. (1994). Phloem sap collection from lettuce (*Lactuca sativa* L.): Chemical comparison among collection methods. *Journal of Chemical Ecology*, **20**, 3191-3206.

van Helden, M., van Heest, H.P.N.F., van Beek, T.A. and Tjallingii, W.F. (1995). Development of a bioassay to test phloem sap samples from lettuce for resistance to *Nasonovia ribisnigri* (Homoptera, Aphididae). *Journal of Chemical Ecology,* **21**, 761-774.

Vankirk, J.R. and AliNiazee, M.T. (1982). Diapause development in the western cherry fruit fly *Rhagoletis indifferens* Curran (Diptera, Tephritidae). *Zeitschrift für angewandte Entomologie,* **93**, 440-445.

Varenhorst, A.J. and O'Neal, M.E. (2012). The response of natural enemies to selective insecticides applied to soybean. *Environmental Entomology*, **41**, 1565-1574.

Vargas, R.R., Troncoso, A.J., Tapia, D.H., Olivares-Donoso, R. and Niemeyer, H.M. (2005). Behavioural differences during host selection between alate virginoparae of generalist and tobacco-specialist *Myzus persicae. Entomologia Experimentalis et Applicata,* **116**, 43-53.

Vasicek, A., La Rossa, F. and Paglioni, y.A. (2000). Aspectos biologicos y poblacionales de *Nasonovia ribisnigri* (Mosley) (Homoptera: aphidoidea) en tres variedades comerciales de lechuga (*Lactuca sativa* L.) en condiciones de laboratorio. *Agricultura Técnica (Chile),* **60**, 350-360.

Vaz Nunes, M. and Hardie, J. (2000a). The effect of temperature on the photoperiodic 'clock' and 'counter' of a Scottish clone of the vetch aphid, *Megoura viciae. Journal of Insect Physiology,* **46**, 727-733.

Vaz Nunes, M. and Hardie, J. (2000b). On critical night lengths and temperature compensation in the photoperiodic response of two geographic clones of the black bean aphid, *Aphis fabae. Physiological Entomology,* **25**, 303-308.

Via, S. (1992). Inducing the sexual forms and hatching the eggs of pea aphids. *Entomologia Experimentalis et Applicata,* **65**, 119-127.

Vorburger, C. (2004). Cold tolerance in obligate and cyclical parthenogens of the peach-potato aphid, *Myzus persicae. Ecological Entomology*, **29**, 498-505.

Vos, P., Simons, G., Jesse, T., Wijbrandi, J., Heinen, L., Hogers, R., Frijters, A., Groenendijk, J., Diergaarde, P., Reijans, M., Fierens-Onstenk, J., de Both, M., Peleman, J., Liharska, T., Hontelez, J. and Zabeau, M. (1998).

The tomato *Mi-1* gene confers resistance to both root-knot nematodes and potato aphids. *Nature Biotechnology,* **16**, 1365-1369.

Völkl, W., Mackauer, M., Pell, J.K. and Brodeur, J. (2007). Predators, parasitoids and pathogens. In: *Aphids as crop pests*, pp. 187-233. Edited by H.F. van Emden and R. Harrington. Wallingford: CABI Publishing.

Wales, S.J., Platt, H.W. and Cattlin, N.D. (2008). Diseases, Pests and Disorders of Potatoes: A Color Handbook. p. 176. New York: Academic Press.

Walley, P.G., Hough, G., Moore, J.D., Carder, J., Elliott, M., Mead, A., Teakle, G., Barker, G., Buchanan-Wollaston, V., Hand, P., Pink, D. and Collier, R. (In preparation). Natural resistance to the currant aphid (*Nasonovia ribisnigri*) in the VeGIN lettuce diversity foundation set. In preparation.

Wang, C.-C. and Furuta, K. (2002). Diapause termination, developmental threshold and thermal requirements of eggs of the maple aphid, *Periphyllus californiensis* Shinji. *Journal of Forest Research,* **7**, 1-6.

Ward, S.A. (1991). Theoretical perspectives on sympatric speciation in aphids (Homoptera: Aphidinea: Aphididae). *Entomologia Generalis,* **16**, 177-192.

Ward, S.A., Leather, S.R. and Dixon, A.F.G. (1984). Temperature prediction and the timing of sex in aphids. *Oecologia,* **62**, 230-233.

Way, M.J. and Banks, C.J. (1964). Natural mortality of eggs of the black bean aphid, *Aphis fabae* Scop., on the spindle tree, *Euonymus europaeus* L. *Annals of Applied Biology,* **54**, 255-267.

Weber, G. (1985). Genetic variability in host plant adaptation of the green peach aphid, *Myzus persicae. Entomologia Experimentalis et Applicata*, **38**, 49-56.

Webster, B., Bruce, T., Pickett, J. and Hardie, J. (2008). Olfactory recognition of host plants in the absence of host-specific volatile compounds. *Communicative and Integrative Biology,* **1**, 167-169.

Weinberg, S.L. and Abramowitz, S.K. (2008). *Statistics using SPSS: An integrative approach*. Second edn. New York: Cambridge University Press.

Weisser, W.W. and Braendle, C. (2001). Body colour and genetic variation in winged morph production in the pea aphid. . *Entomologia Experimentalis et Applicata,* **99**, 217-223.

Wellings, P.W., Leather, S.R. and Dixon, A.F.G. (1980). Seasonal variation in reproductive potential: a programmed feature of aphid life cycles. *Journal of Animal Ecology,* **49**, 975-985.

Wells, M.L., McPherson, R.M., Ruberson, J.R. and Herzog, G.A. (2000). Effect of fungicide application on activity of *Neozygites fresenii*

(Entomophthorales: Neozygitacaea) and cotton aphid (Homoptera: Aphididae) suppression. *Journal of Economic Entomology*, **93**, 1118-1126.

Wilding, N. (1981). The effect of introducing aphid-pathogenic Entomophthoraceae into field populations of *Aphis fabae*. *Annals of Applied Biology*, **99**, 11-23.

Wilding, N. and Perry, J.N. (1980). Studies on *Entomophthora* in populations of *Aphis fabae* on field beans. *Annals of Applied Biology*, **94**, 367–378.

Wilkinson, T.L. and Douglas, A.E. (2003). Phloem amino acids and the host plant range of the polyphagous aphid, *Aphis fabae*. *Entomologia Experimentalis et Applicata*, **106**, 103-113.

Williams, I.S. and Dixon, A.F.G. (2007). Life cycles and polymorphisms. In: *Aphids as crop pests*, pp. 69-86. Edited by H.F. van Emden and R. Harrington. Wallingford: CABI Publishing.

Winkler, K., Wäckers, F., Bukovinszkine-Kiss, G. and van Lenteren, J. (2006). Sugar resources are vital for *Diadegma semiclausum* fecundity under field conditions. *Basic and Applied Ecology*, **7**, 133-140.

Wipperfűrth, T. and Mittler, T.E. (1986). A method for hatching the eggs of aphid *Schizaphis graminum*. *Entomologia Experimentalis et Applicata*, **42**, 57-61.

Woiwod, I.P., Tatchell, G.M. and Barrett, A.M. (1984). A system for the rapid collection, analysis and dissemination of aphid-monitoring data from suction traps. *Crop Protection*, **3**, 273-288.

Wolfgang, W.W. and Braendle, C. (2001). Body colour and genetic variation in winged morph production in the pea aphid. . *Entomologia Experimentalis et Applicata*, **99**, 217-223.

Wyatt, I.J. and White, P.F. (1977). Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *Journal of Applied Ecology*, **14**, 757-766.

Wyss, E., Villiger, M. and Müller-Schärer, H. (1999). The potential of three native insect predators to control the rosy apple aphid, *Dysahis plantaginea*. *BioControl*, **44**, 171-182.

Wäckers, F.L. (2001). A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *Journal of Insect Physiology*, **47**, 1077-1084.

Wöhrmann, K. and Tomiuk, J. (1988). Life cycle strategies and genotypic variability in populations of aphids. *Journal of Genetics*, **67**, 43-52.

Yencho, G.C., Cohen, M.B. and Byrne, P.F. (2000). Applications of tagging and mapping insect resistance loci in plants. *Annual Review of Entomology*, **45**, 393-422.

Zera, J.Z. and Denno, R.F. (1997). Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology*, **42**, 207-230.

Zhang, Y., Wu, K., Wyckhuys, K.A.G. and Heimpel, G.E (2009). Trade-offs between flight and fecundity in the soybean aphid (Hemiptera: Aphididae). *Journal of Economic Entomology*, **102**, 133-138.